horticulturae

MDPI

Article

Comparative Analysis of Glucosinolate and Phenolic Compounds in Green and Red Kimchi Cabbage (*Brassica rapa* L. ssp. *pekinensis*) Hairy Roots after Exposure to Light and Dark Conditions

Sook Young Lee [1], Haejin Kwon [2], Jae Kwang Kim [3], Chang Ha Park [4], Ramaraj Sathasivam [2,*] and Sang Un Park [2,5,*]

1 Marine Bio Research Center, Chosun University, 61-220 Myeongsasimni, Sinji-myeon, Wando-gun 59146, Republic of Korea
2 Department of Crop Science, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 34134, Republic of Korea
3 Division of Life Sciences and Convergence Research Center for Insect Vectors, College of Life Sciences and Bioengineering, Incheon National University, Yeonsu-gu, Incheon 22012, Republic of Korea
4 Department of Biological Sciences, Keimyung University, Dalgubeol-daero 1095, Dalseo-gu, Daegu 42601, Republic of Korea
5 Department of Smart Agriculture Systems, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 34134, Republic of Korea
* Correspondence: ramarajbiotech@gmail.com (R.S.); supark@cnu.ac.kr (S.U.P.)

Abstract: *Brassica rapa* L. ssp. *pekinensis* (Lour.) Hanelt (kimchi cabbage) is a major vegetable cultivated in Korea, and its hairy roots (HRs) are rich in glucosinolates and phenolic compounds. This study aimed to induce HRs from cotyledon explants via the transformation of the *Agroacterium rhizogenes* strain R1000 and examine the glucosinolate and phenolic compounds present in the HRs of two kimchi cabbage (green and red) cultivars after exposure to 16 h light/8 h dark conditions (photosynthetic photon flux density of 54.6 µmol m^{-2} s^{-1}) and continuous dark conditions. The highest HR production was achieved in the green kimchi cabbage grown under dark conditions (0.37 ± 0.01 DW g/30 mL). The highest glucosinolate and phenolic contents were neoglucobrassicin and catechin hydrate, which were highest in the green kimchi HRs grown under dark (GKHD) conditions (5268.29 ± 292.84 µg/g DW) and green HRs grown under light (GKHL) conditions (203.49 ± 4.70 µg/g DW), respectively. A heat map showed that the red kimchi HRs grown under dark conditions (RKHD) and the GKHL condition accumulated the highest glucosinolate and phenolic contents. Principal component (PCA) and partial least-squares discriminant (PLS-DA) analyses of the 13 identified metabolites showed a clear separation. According to a variable importance in projection (VIP) analysis, quercetin was the most important metabolite, leading to a clear separation. The most suitable conditions for enhancing the glucosinolate and phenolic contents were the GKHD and GKHL conditions, respectively, whereas both compounds were enhanced in the RKHD condition. HRs cultures cultivated under light and dark conditions are a promising method to enhance the production of specific health-promoting bioactive metabolites, which might be helpful in the pharmaceutical and nutraceutical industries.

Keywords: *Agrobacterium rhizogenes*; *Brassica rapa* L. ssp. *pekinensis*; hairy root; glucosinolate; phenolic compounds

Citation: Lee, S.Y.; Kwon, H.; Kim, J.K.; Park, C.H.; Sathasivam, R.; Park, S.U. Comparative Analysis of Glucosinolate and Phenolic Compounds in Green and Red Kimchi Cabbage (*Brassica rapa* L. ssp. *pekinensis*) Hairy Roots after Exposure to Light and Dark Conditions. *Horticulturae* **2023**, *9*, 466. https://doi.org/10.3390/horticulturae9040466

Academic Editor: Wajid Zaman

Received: 10 March 2023
Revised: 4 April 2023
Accepted: 5 April 2023
Published: 7 April 2023

1. Introduction

Brassica rapa L. ssp. *pekinensis* (Lour.) Hanelt, otherwise called Kimchi cabbage, belongs to the Brassicaceae family, and it is the main ingredient of traditional Korean foods. It is prepared through a series of fermentation processes [1]. In kimchi cabbage, several phenolic compounds with antioxidant activity and flavonoids have been identified [2,3].

In addition, it contains aliphatic glucosinolates, aromatic glucosinolates, and relatively high amounts of indolic glucosinolates, a precursor to indole-3-carbinol, which chemically prevents cancer [4,5].

Different varieties of kimchi cabbage have been produced through introgression breeding techniques. Among these, Xie et al. [6] introduced the red phenotypic kimchi cabbage variety crossed with the red *Brassica juncea* using the embryo rescue procedure. In another study, reddish purple and red kimchi cabbage were obtained via interspecific crossing between red and kimchi cabbage [7]. The color of red kimchi cabbage is reddish purple, and it has an abundant anthocyanin content. Due to its attractive color and high antioxidant properties, it is popularly used in salads [7].

Glucosinolates (GSLs) are secondary metabolites (SMs) containing nitrogen and sulfur that play vital roles in human health and plant defense mechanisms [8]. To date, more than 200 GSLs have been detected in plants, mainly in the Brassica family [9]. GSLs are derivatives of amino acids—phenylalanine, alanine, methionine, isoleucine, tryptophan, tyrosine, and valine—and are separated into three major groups corresponding to their amino acid precursors, namely aliphatic, aromatic, and indolic GSLs [9,10].

Phenolic compounds are SMs composed of aromatic rings containing one or more hydroxyl groups, and they are very common in plants [11]. They can be classified into flavonoids (anthocyanidins, flavanones, flavan-3-ols, flavonols, flavones, isoflavones, and others) and non-flavonoids (hydroxycinnamates, stilbenes, phenolic acids, and others) based on their arrangement and number of carbon atoms [12]. In addition, phenolic compounds play a vital role in human health, with anti-inflammatory, antibacterial, and antioxidant actions [13–15].

Agrobacterium rhizogenes infects higher plants and is responsible for the growth of hairy root (HR) disease, mainly in dicotyledonous plants and a few monocotyledonous plants [16]. HR cultures have been developed from several plant species through *A. rhizogenes* transformation to enhance SM production [17–19]. The phenotypic pattern of HRs is defined by genetic stability, a lack of geotropism, hormone-independent growth, lateral branching, and the ability to produce specific SMs. The main benefit of HRs is that they often show a better biosynthetic capacity for SM production than their parent plants [20].

Light is a major factor in the growth and production of SMs in HR cultures [21]. Several studies have shown that light affects the biosynthesis of SMs in HR cultures [22–24]. In this respect, the purpose of this study was to develop HRs of green and red Kimchi cabbage using an *A. rhizogenes*-mediated HR transformation and analyze their glucosinolates and phenolic compounds after exposure to light and dark conditions. These results will provide basic information for further bio-engineering research to increase the glucosinolates and phenylpropanoid contents in the HRs of kimchi cabbage varieties.

2. Materials and Methods

2.1. Seed Sterilization and Germination

Seeds of green (cv. CR carotene) and red (cv. Ppalgang 3) Kimchi cabbage were purchased from the Kwonnong Seed Company (Cheongju, Republic of Korea). The seeds were surface-sterilized with 70% (*v/v*) ethanol for 30 s and then soaked with a 4% (*v/v*) NaClO solution containing one drop of a Tween 20 solution for 10 min. The seeds were then washed 5–6 times with sterilized distilled water. The sterilized seeds were taken to a clean bench and dried with sterilized tissue paper. Seven seeds were placed on a Petri dish containing solidified hormone-free 1/2 Murashige and Skoog Basal Medium (MS) (Kisan Bio, Seoul, Republic of Korea) [25] containing 0.8% (*w/v*) plant agar. The Petri dishes were kept in a growth chamber under 16 h light/8 h dark photoperiod cycles at 25 °C until seedlings were established. The seedlings were then moved into solidified hormone-free 1/2 MS medium after two to three weeks.

2.2. Growth of *A. rhizogenes*

Wild-type *A. rhizogenes* strain R1000 bacterial cells were cultured in a flask containing 30 mL of Luria–Bertani (LB) liquid medium (Ambrothia, Daejeon, Republic of Korea) and incubated in a continuous rotary shaker (Hanbaek scientific Co., HB-201SF, Republic of Korea) at 28 °C for 20 h at 200 rpm in dark conditions. The bacterial cell suspension was centrifuged (Mega 21R, Hanil, Incheon, Republic of Korea) at 3000× *g* for 10 min at 4 °C to collect the cells and resuspended with $\frac{1}{2}$ MS liquid medium to maintain a final cell density of OD_{600} = 1.0 for plant inoculation.

2.3. Establishment of HR Cultures

From the 10-day-old kimchi cabbage cultivar seedlings, the cotyledons were excised and cut into tiny pieces (about 0.5 × 0.5 cm) with a sterile blade. The sliced explants were co-cultured with the bacterial suspension for 10–15 min. After drying with sterile tissue paper, the co-cultured explants were placed in Petri dishes containing hormone-free 1/2 MS medium with 0.8% (*w/v*) agar and were incubated for two days at 25 °C in the dark. The explants were removed after 2 days of co-cultivation, washed 5–6 times in sterile distilled water, and then moved to a hormone-free 1/2 MS medium containing 500 mg/L cefotaxime (GoldBio, St. Louis, MO, USA) for HR induction [26]. From the wounded site, several HRs emerged within 3–4 weeks, and the HRs were excised, transferred to the hormone-free 1/2 MS medium containing 500 mg/L cefotaxime, and incubated at 25 °C in dark conditions. This procedure was repeated 3–4 times.

2.4. HR Culture under Light and Dark Conditions

Approximately 4 g of HRs of both green and red kimchi cabbage were transferred to 30 mL of the hormone-free $\frac{1}{2}$ MS liquid medium and incubated on a shaker (Hanbaek scientific Co., HB-201SLI, Republic of Korea) at 110 rpm for 2 weeks at 25 °C. Each type of HR was cultured in six flasks; three flasks were placed under 16 h light/8 h dark conditions (photosynthetic photon flux density of 54.6 µmol m^{-2} s^{-1}), and the other three flasks were placed under continuous dark conditions [27]. After 2 weeks, HRs were harvested and dried using a freeze dryer (HyperCool HC3055, Hanil Scientific Inc., Gimpo, Republic of Korea) for at least 3 days to measure the dry weight (DW) of the HRs and to analyze the secondary metabolites.

2.5. Extraction and Analysis of Glucosinolate Compounds

Glucosinolates were extracted and analyzed as described by Sathasivam et al. [10,28], with slight modifications. Approximately 100 mg of freeze-dried green and red kimchi was dissolved with 4.5 mL of 70% methanol (*v/v*), thoroughly mixed by vortexing (KMC-1300 V, Vision Scientific Co., Ltd., Daejeon-Si, Republic of Korea), incubated at 70 °C for 5 min, and centrifuged at 14,000 rpm for 10 min at 4 °C. A diethylaminoethanol Sephadex A-25 column (GE Healthcare, Uppsala, Sweden) was used to separate and filter the upper layer, and the column was washed with 3 mL of autoclaved distilled water. For the desulfation of the eluted mixture, 75 µL of purified arylsulfatase was added, and it was kept at room temperature overnight. After the overnight incubation, the mixture was eluted with 0.5 mL of ultrapure water, filtered, and sterilized using a 0.22 µm PTFE syringe filter (Sterlitech Corp., Kent, WA, USA) before being injected for high-performance liquid chromatography (HPLC) analysis. The HPLC model, column conditions, operating conditions, and gradient program are shown in Table S1. The glucosinolate content was analyzed, measured, and quantified according to the method described by Sathasivam et al. [28].

2.6. Extraction and Analysis of Phenolic Compounds

Phenolic compounds were extracted and analyzed following the protocol described by Sathasivam et al. [10,28]. To 100 mg of freeze-dried green and red kimchi samples, 3 mL of an 80% MeOH (*v/v*) solution was added. The samples were then vortexed for 1 min and immediately sonicated at 37 °C for 1 h. At 4 °C, the mixture was centrifuged for 15 min at

10,000 rpm. The resulting supernatants were filtered through a 0.45 µm PTFE syringe filter (Millipore, Bedford, MA, USA) before being injected for HPLC analysis. The HPLC model, column conditions, operating conditions, and gradient program are shown in Table S1. The phenolic content was measured and quantified with reference to a corresponding standard calibration curve [28].

2.7. Statistical Analysis

The statistical analysis of the data was performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). The data were calculated as the means ± standard deviations of three replicates, and Duncan's multiple range test (DMRT) was used to determine whether there was a significant difference ($p < 0.05$) among the means. PCA, PLS-DA, a heat map analysis, a Pearson correlation analysis, and a VIP analysis of 13 metabolites identified in the GKHL, GKHD, RKHL, and RKHD conditions were performed using MetaboAnalyst 5.0. Autoscaling was carried out as a preprocessing approach for the data set.

3. Results

3.1. Growth Patterns of Kimchi Cabbage HRs under Light and Dark Conditions

The growth of the green kimchi cabbage HRs grown under light (GKHL) conditions, green kimchi cabbage HRs grown under dark (GKHD) conditions, red kimchi cabbage HRs grown under light (RKHL) conditions, and red kimchi cabbage HRs grown under dark (RKHD) conditions ranged from 0.37 to 0.27 g/30 mL (Figure 1). The highest HR growth was obtained in the GKHD condition (0.37 ± 0.01 DW g/30 mL), followed by the RKHD condition (0.33 ± 0.01 g/30 mL), whereas the GKHL condition (0.31 ± 0.02 DW g/30 mL) and the RKHL condition (0.27 ± 0.01 g/30 mL) showed the lowest growth patterns. The growth was reduced by 1.12-, 1.19-, and 1.37-fold by the RKHD condition, GKHL condition, and RKHL condition, respectively, when compared to the GKHD condition. These findings suggest that HRs grown under dark conditions are more suitable for the growth of green and red kimchi cabbage compared to light conditions.

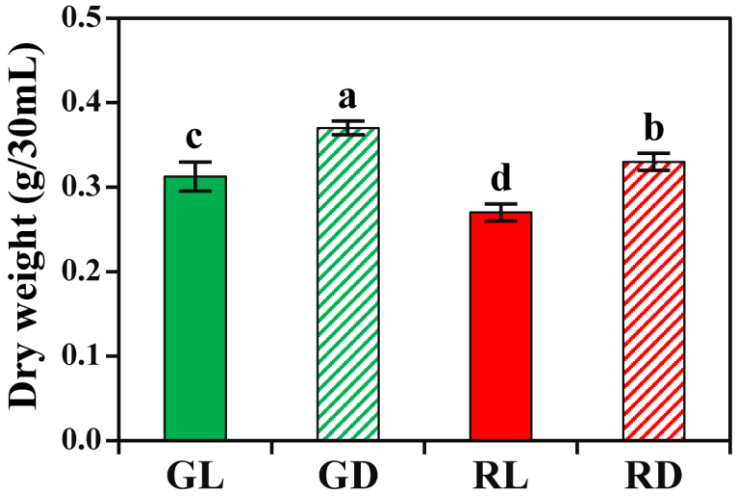

Figure 1. Growth of green and red kimchi cabbage HRs grown under light and dark conditions. Samples were harvested after 10 days of HR growth. For light and dark conditions, HRs were placed under 16 h light/8 h dark conditions and continuous dark conditions, respectively. Significant differences exist between mean values with distinct letters ($p < 0.05$, ANOVA and DMRT). GL—green kimchi cabbage grown under light conditions; GD—green kimchi cabbage grown under dark conditions; RL—red kimchi cabbage grown under light conditions; RD—red kimchi cabbage grown under dark conditions.

3.2. Glucosinolate Accumulation in Response to Light and Dark Conditions

The glucosinolate accumulation in green and red kimchi cabbage HRs was analyzed using HPLC. Five glucosinolate compounds, i.e., glucobrassicin, glucoerucin, 4-methoxyglucobrassicin, gluconasturtiin, and neoglucobrassicin, were detected in the GKHL, GKHD, RKHL, and RKHD conditions (Figure 2). Their contents were significantly different under light and dark conditions. In the GKHL, GKHD, RKHL, and RKHD conditions, neoglucobrassicin comprised the largest portion of the quantified glucosinolate content. Neoglucobrassicin was more highly accumulated in the GKHD condition than in the other kimchi cabbage HRs grown under light and dark conditions. Similarly, the GKHD condition also enhanced gluconasturtiin production. The glucobrassicin content of the kimchi cabbage HRs was more affected by the light and dark conditions when compared to the other four glucosinolate compounds. Compared with the RKHD condition, the amount of glucobrassicin was 2.72-, 2.61-, and 2.16-fold lower in GKHL, RKHL, and GKHD conditions, respectively. In addition, the levels of glucoerucin and 4-methoxyglucobrassicin were also highest in the RKHD condition. The glucoerucin contents were 1.27-, 1.22-, and 1.27-fold lower in the GKHL, GKHD, and RKHL conditions, respectively, than in the RKHD condition. Moreover, the level of 4-methoxyglucobrassicin was highest in the RKHD condition, which was 1.19-, 1.14-, and 1.09-fold higher compared to the GKHL, RKHL, and GKHD conditions, respectively. The total glucosinolate contents ranged from 6884.55 to 8864.58 μg/DW in response to different HRs of kimchi cabbages grown under light and dark conditions. In particular, the total glucosinolate content of the RKHD condition was the highest, which was 1.29-fold higher than that of the RKHL condition. The total amounts of glucosinolate in the GKHL and GKHD conditions were 1.29- and 1.06-fold lower than in the RKHD condition, respectively. This overall result showed that dark conditions enhance glucosinolate accumulation in the HRs of kimchi cabbage.

3.3. Phenolic Accumulation in Response to Light and Dark Conditions

The phenolic accumulation in green and red kimchi cabbage HRs was analyzed using HPLC. Seven different phenolic compounds, i.e., caffeic acid, catechin hydrate, chlorogenic acid, *p*-coumaric acid, epicatechin, quercetin, trans-cinnamic acid, and 4-hydroxybenzoic acid, were detected in the GKHL, GKHD, RKHL, and RKHD conditions (Figure 3). All phenolic compounds showed differential accumulation in the GKHL, GKHD, RKHL, and RKHD conditions. The total phenolic contents ranged from 250.53 to 289.22 μg/g DW in response to different HRs of kimchi cabbages grown under light and dark conditions. The total phenolic contents in the GKHL, RKHL, and RKHD conditions did not show any significant differences, whereas they were slightly decreased in the GKHD condition. The total phenolic content in the GKHD condition was 1.15-, 1.14-, and 1.13-fold lower than those of the GKHL, RKHD, and RKHL conditions, respectively. In particular, catechin hydrate comprised the largest portion of the quantified phenolic content in the GKHL, GKHD, RKHL, and RKHD conditions. Among the two HRs grown under different conditions, most of the individual phenolic compounds, such as trans-cinnamic acid, caffeic acid, catechin hydrate, chlorogenic acid, and *p*-coumaric acid, were highest in the GKHL condition. The quercetin contents were significantly higher in the RKHL and RKHD conditions compared to those of the GKHL and GKHD conditions. In contrast, the 4-hydroxybenzoic acid contents were significantly higher in the GKHL and GKHD conditions than in the RKHL and RKHD conditions. Interestingly, the trans-cinnamic acid content was significantly higher in the GKHL condition, whereas it was not detected in the RKHD condition. The trans-cinnamic acid contents were 1.93- and 2.32-fold lower in the GKHD and RKHL conditions, respectively, than in green kimchi cabbage HRs grown under light conditions. This result shows that most of the individual and total phenolic contents were highest in the GKHL condition.

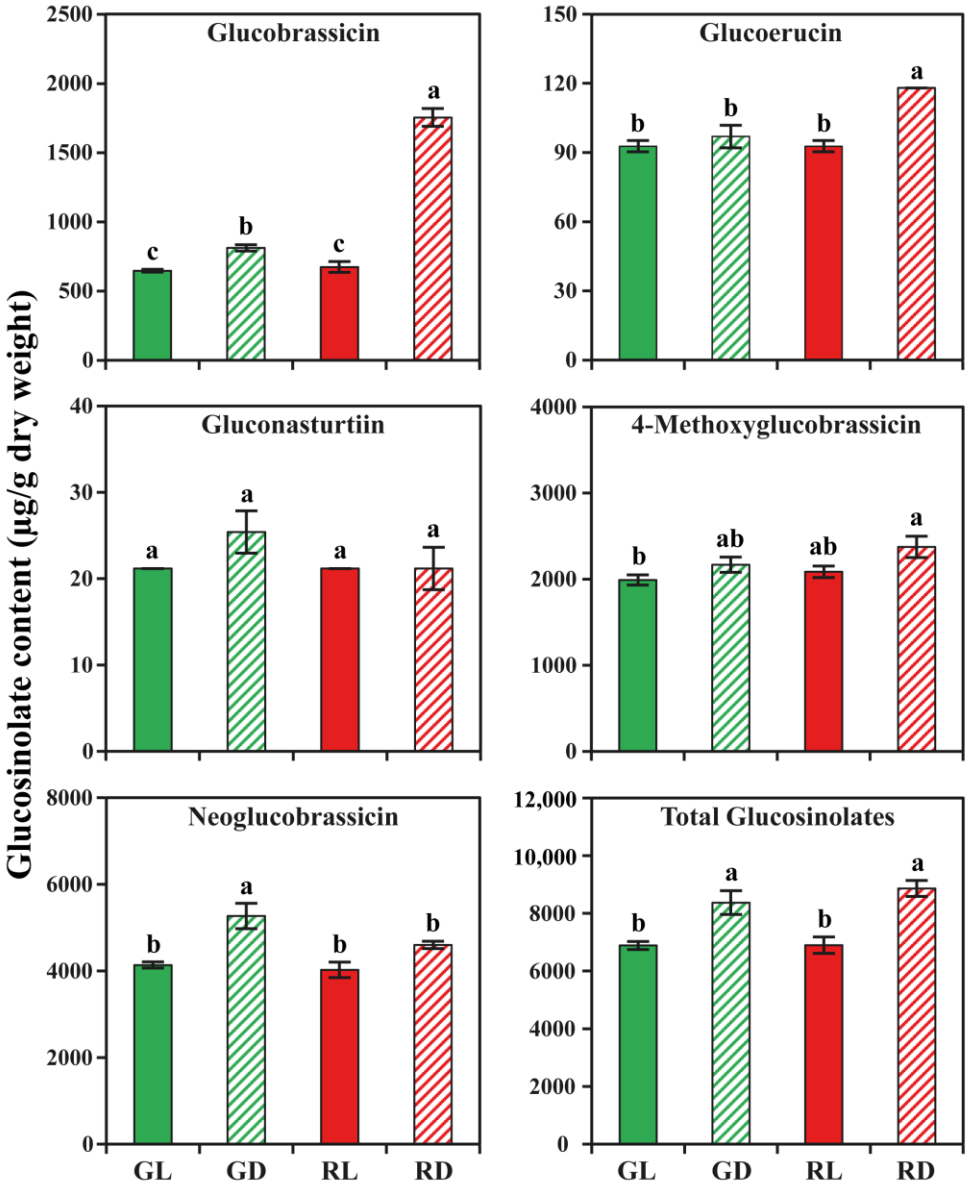

Figure 2. Effect of light and dark conditions on glucosinolate compound (μg/g DW of the HR cultures) accumulation in HR cultures of green and red kimchi cabbage. Samples were harvested after 10 days of HR growth. Significant differences exist between mean values with distinct letters ($p < 0.05$, ANOVA and DMRT). GL—green kimchi cabbage grown under light conditions; GD—green kimchi cabbage grown under dark conditions; RL—red kimchi cabbage grown under light conditions; RD—red kimchi cabbage grown under dark conditions.

3.4. Metabolic Profiling of Identified Metabolites in Response to Light and Dark Conditions

In total, five individual glucosinolate compounds and eight phenolic compounds were identified and quantified in samples from the GKHL, GKHD, RKHL, and RKHD conditions using HPLC. A heat map showed that most of the individual glucosinolate and phenolic compounds were significantly higher in the RKHD and the GKHL conditions, respectively (Figure 4). The heat map was divided into two major clusters, namely cluster 1 and cluster 2, but they were further subdivided into subclusters 1-1, 1-2, 2-1, and 2-2. Clusters 1-1, 2-1, and 2-2 were further subgrouped into two subclusters, namely 1-1a and 1-1b, 2-1a and 2-1b, and 2-2a and 2-2b, respectively. Cluster 1 contained the metabolites abundantly present in the GKHL condition, whereas cluster 2 was separated based on the lowest metabolites present in the GKHL condition. Cluster 1-1 was further separated into two

clusters, namely 1-1a and 1-1b. Cluster 1-1a was separated based on the highest amounts of phenolic compounds present in the HRs exposed to the light conditions (GKHL and RKHL conditions), whereas cluster 1-1b formed a group based on the phenolic compounds rich in specific cultivars (GKHL and GKHD conditions). In addition, most of the metabolites identified in the GKHL, GKHD, RKHL, and RKHD conditions were separated into distinct groups (e.g., caffeic acid was significantly abundant in the GKHL, RKHL, and RKHD conditions (cluster 1-2)). Cluster 2-1 was also separated into a distinct group. Cluster 2-1a formed a cluster based on the abundant metabolites present in specific cultivars (GKHL and GKHD conditions), whereas 2-1b was separated based on the metabolites that were abundant in specific conditions (RKHD and GKHD conditions). Cluster 2-2 was separated based on the metabolites that were significantly abundant in the RKHD conditions. This cluster was divided into two subgroups, namely 2-2a and 2-2b. Subgroups 2-2a and 2-2b formed a group based on the metabolites that were significantly abundant in different cultivars grown under different conditions (RKHD and GKHD dark conditions). Regarding the glucosinolates, the metabolites present in the HRs of two cultivars grown under light and dark conditions were grouped in clusters 2-1b and 2-2a (gluconasturtiin, neoglucobrassicin, 4-methoxyglucobrassicin, glucoerucin, and glucobrassicin), whereas the phenolic compounds and metabolites from the HRs of two cultivars grown under light and dark conditions were separated into three groups (Figure 4). Cluster 1 consisted of catechin hydrate, *p*-coumaric acid, chlorogenic acid, caffeic acid, and trans-cinnamic acid. In group 2-la, 4-hydroxybenzoic acid formed a separate cluster, whereas in group 2-2b, epicatechin and quercetin were clustered. Significantly higher ($p \leq 0.05$) levels of glucosinolate and phenolic compounds in the GKHL, GKHD, RKHL, and RKHD conditions are shown in Figure S1, which demonstrates that unique metabolite signature characteristics were found in the GKHL, GKHD, RKHL, and RKHD conditions.

According to the PCA findings, PC1 and PC2 showed 47.5% and 23.6% of the variance, respectively (Figure 5). The PCA showed a clear separation among the different HRs grown under light and dark conditions. This clear separation among the different cultivars grown under light and dark conditions might be due to 4-methoxyglucobrassicin, glucoerucin, glucobrassicin, epicatechin, and quercetin, and their associated eigenvector values were −0.32494, −0.29928, −0.29511, −0.26775, and −0.25909, respectively. Those of trans-cinnamic acid, *p*-coumaric acid, catechin hydrate, chlorogenic acid, and 4-hydroxybenzoic acid were 0.34956, 0.34201, 0.27291, 0.25167, and 0.19125, respectively (Figure 5A). In addition, a PLS-DA was performed to maximize the separation between different kimchi cabbage HRs grown under light and dark conditions. The PLS-DA model showed a clear separation between the different HRs grown under light and dark conditions, which were 24.9% (PC1) and 39.5% (PC2). The PLS-DA also showed a clear separation between the HRs grown under light and dark conditions. This supports the PCA results. This clear separation might be due to trans-cinnamic acid, 4-hydroxybenzoic acid, neoglucobrassicin, gluconasturtiin, and chlorogenic acid, and their associated eigenvector values were −0.49375, −0.34139, −0.3139, −0.30284, and −0.26393, respectively. Those of quercetin, glucobrassicin, glucoerucin, epicatechin, and 4-methoxyglucobrassicin were 0.46467, 0.27146, 0.21669, 0.21551, and 0.12372, respectively (Figure 5B). In addition, quercetin, 4-hydroxybenzoic acid, and neoglucobrassicin were the most significant metabolites in the prediction, according to the VIP analysis, which led to a clearer separation between the green and red kimchi cabbage than the different conditions (Figure 6). The green and red kimchi cabbage HRs grown under dark conditions showed a clear separation in both the PCA and PLS-DA compared to the GKHL and RKHL conditions. This might have been because the glucosinolate contents were significantly higher in the HRs grown under dark conditions than under light conditions. However, the GKHL and RKHL conditions showed a slightly closer group than the GKHD and RKHD conditions. This was because the total glucosinolate and total phenolic contents were similar in both cultivars grown under light conditions. From these results, for the enhancement of the glucosinolate and phenolic contents in the HRs of both kimchi cabbage cultivars, the dark and light conditions were the most suitable, respectively.

This supports the heat map results, which showed that most of the individual glucosinolate and phenolic compounds were highest in kimchi cabbage HRs grown under dark and light conditions, respectively.

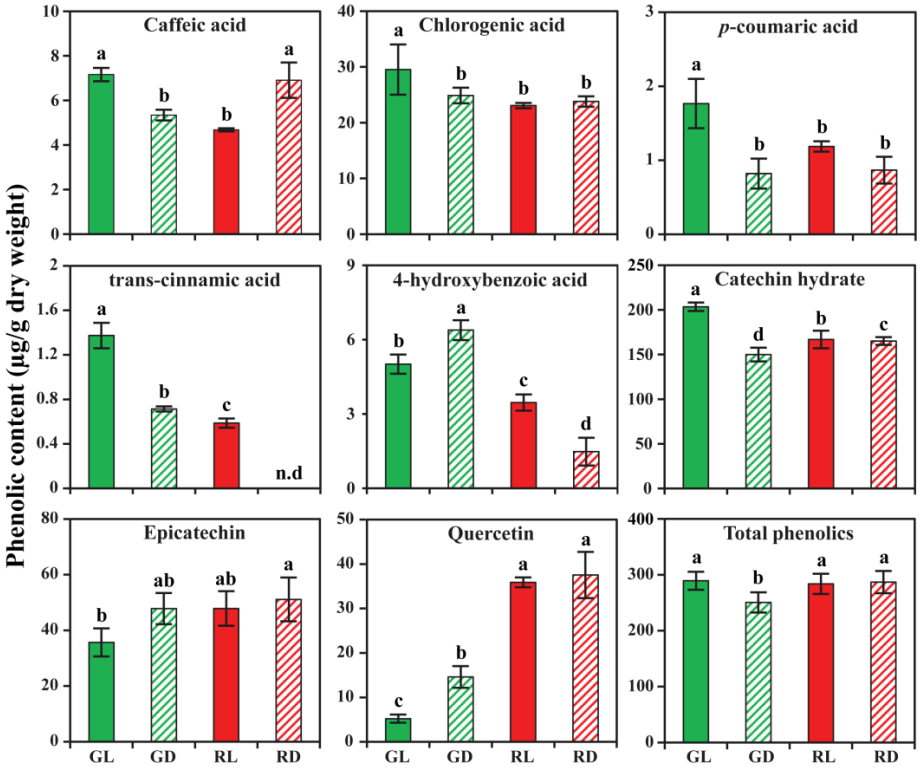

Figure 3. Effect of light and dark treatments on the phenolic compound (μg/g DW of the HR cultures) accumulation in HR cultures of green and red kimchi cabbage. Samples were harvested after 10 days of HR growth. Significant differences exist between mean values with distinct letters ($p < 0.05$, ANOVA and DMRT). n.d.—not detected. GL—green kimchi cabbage grown under light conditions; GD—green kimchi cabbage grown under dark conditions; RL—red kimchi cabbage grown under light conditions; RD—red kimchi cabbage grown under dark conditions.

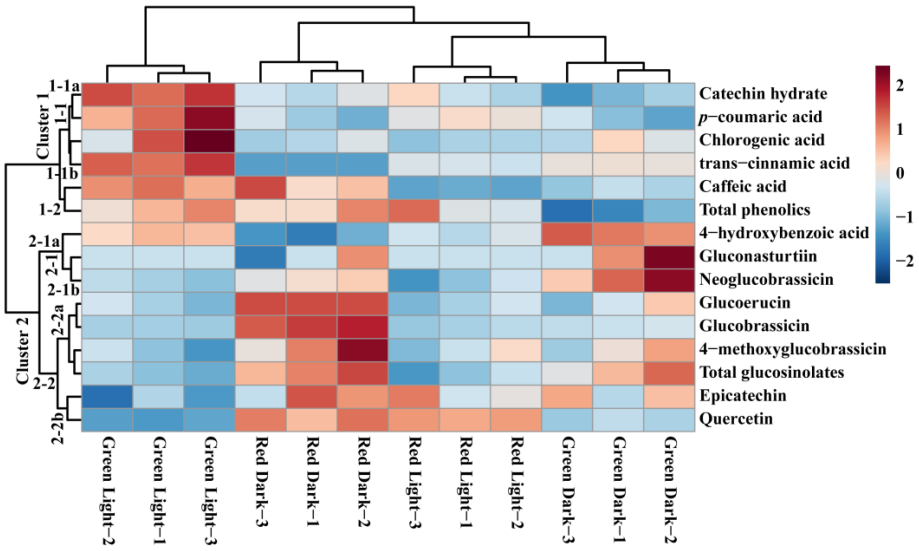

Figure 4. Heatmap showing the differences in the relative metabolite contents in the HRs of green and red kimchi cabbage after exposure to light and dark conditions. Blue denotes a reduction, whereas red indicates an increase in metabolite concentration.

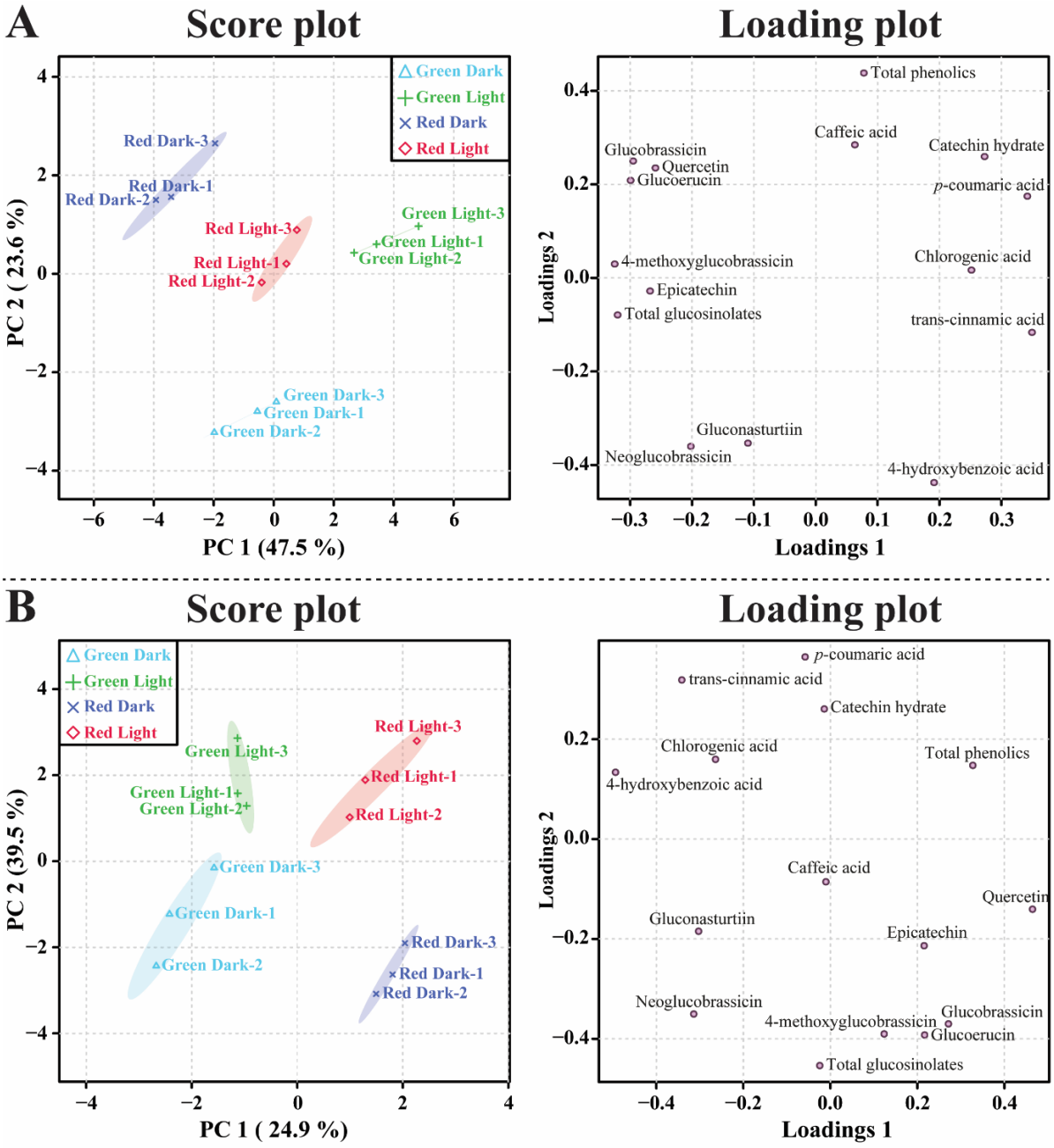

Figure 5. Score and loading plots of the PCA (**A**) and PLS-DA (**B**) models of the metabolites found in the HRs of green and red kimchi cabbage after exposure to light and dark conditions.

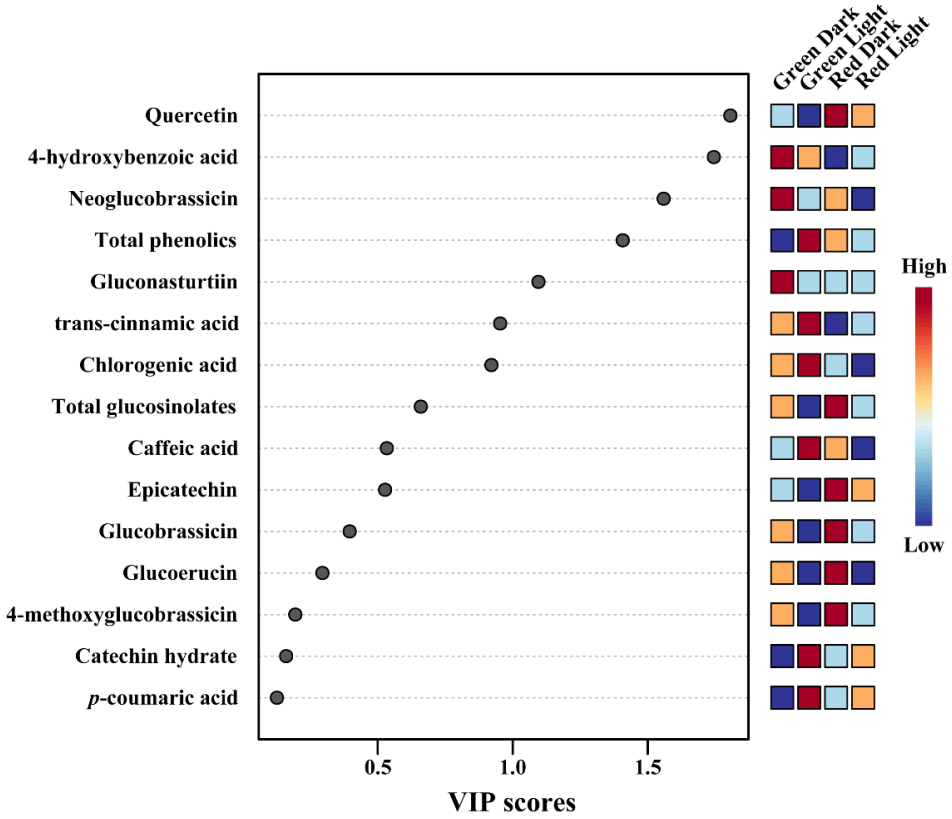

Figure 6. The main components separating the HRs of green and red kimchi cabbage after exposure to light and dark conditions, based on the VIP scores obtained using the PLS-DA model.

A correlation matrix analysis of 13 metabolites identified after exposing green and red HRs to light and dark conditions (Figure 7) was clustered into two main groups that consisted of both individual glucosinolates and phenolic compounds based on positive correlations. The most significant metabolite, according to VIP analysis, was quercetin, which showed positive correlations with epicatechin (r = 0.55481, p = 0.061166), glucoerucin (r = 0.51565, p = 0.086173), glucobrassicin (r = 0.57067, p = 0.052656), and 4-methoxyglucobrassicin (r = 0.49907, p = 0.098581), whereas it showed negative correlations with most of the individual glucosinolate and phenolic compounds, namely chlorogenic acid, gluconasturtiin, neoglucobrassicin, caffeic acid, catechin hydrate, *p*-coumaric acid, 4-hydroxybenzoic acid, and trans-cinnamic acid. Among the identified individual glucosinolate compounds, neoglucobrassicin showed the highest content and showed strong positive correlations with gluconasturtiin, epicatechin, glucoerucin, glucobrassicin, 4-methoxyglucobrassicin, and 4-hydroxybenzoic acid, whereas catechin hydrate showed the highest content when compared to the other individual phenolic compounds and showed positive correlations with caffeic acid (r = 0.57526, p = 0.050358), *p*-coumaric acid (r = 0.80613, p = 0.0015418), chlorogenic acid (r = 0.65078, p = 0.021916), and trans-cinnamic acid (r = 0.70098, p = 0.011095). However, catechin hydrate showed a negative correlation with several compounds, namely gluconasturtiin, neoglucobrassicin, epicatechin, quercetin, glucoerucin, glucobrassicin, 4-methoxyglucobrassicin, and 4-hydroxybenzoic acid.

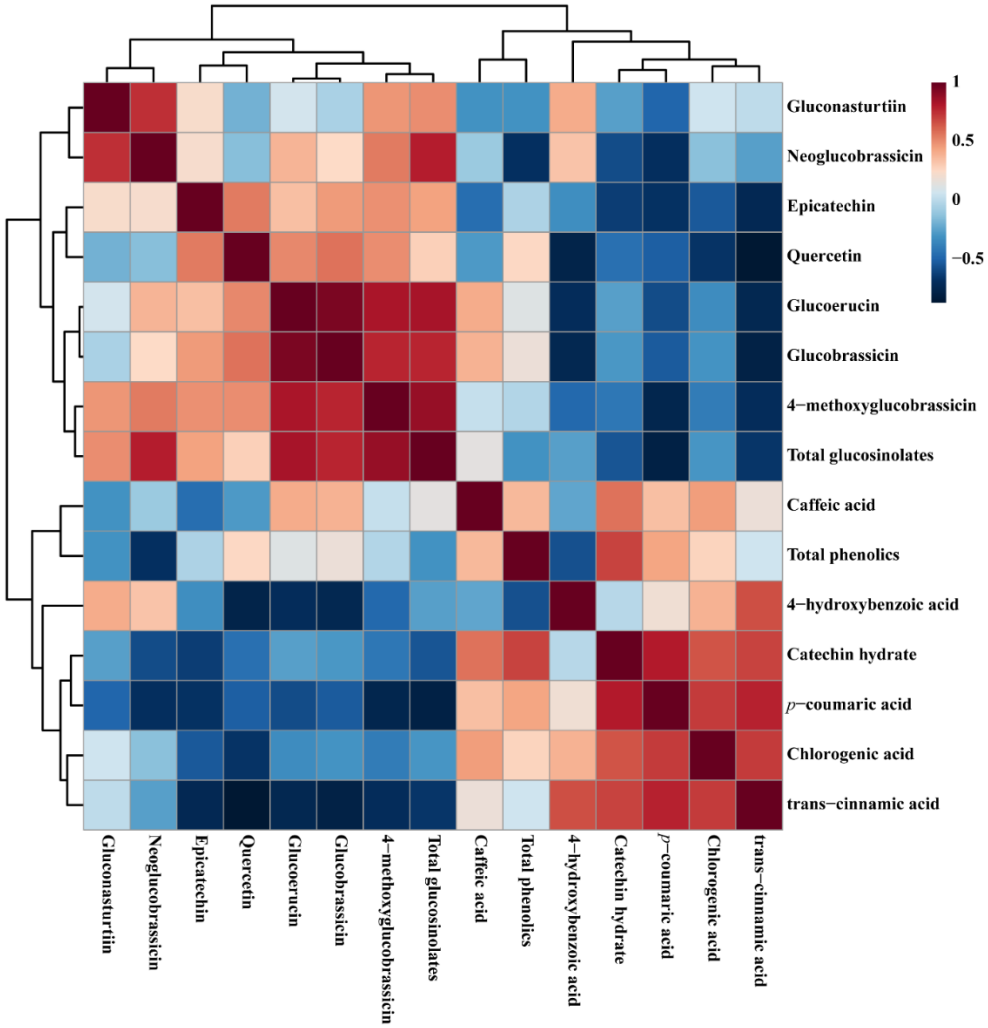

Figure 7. Correlation matrix showing the relationships between the metabolites found in the HRs of green and red kimchi cabbage grown under light and dark conditions. The intensity of the blue or red color, as shown on the color scale, denotes the correlation coefficient value for each colored box, which indicates Pearson's correlation coefficient for a pair of metabolites.

4. Discussion

HRs can grow vigorously on a medium without adding hormones to produce a significant amount of valuable SMs [29,30]. In this study, dark conditions enhanced the DW of HRs in both green and red kimchi cabbage. A similar result was obtained in a previous study in which the HRs of green and purple *Ocimum basilicum* were grown under light and dark conditions; the results showed that the highest growth rate was achieved under dark conditions [31]. Therefore, to enhance HR growth in plant species, dark conditions are the most appropriate.

Previously, several studies reported that HRs accumulate significant phenolic and flavonoid contents [32–34]. For instance, HR cultures of *Dracocephalum moldavica* and *Momordica charantia* showed higher accumulations of total flavonoid and phenolic contents than non-transformed roots [33,34]. Similarly, an HR culture of *Beta vulgaris* accumulated 20-fold higher levels of phenolic compounds than a control culture [35]. A previous study demonstrated that higher glucosinolate concentrations were achieved in the roots than in the shoots at an identical developmental stage in cabbage and radish [36]. In transgenic HRs, 4-hydroxyglucobrassicin, gluconasturtiin, 4-methoxyglucobrassicin, glucobrassicin, and neoglucobrassicin were highly present in watercress [37]. A similar result was obtained in this study; these compounds were also rich in the HRs of green and red kimchi

cabbage. Kim et al. [38] reported that *Fagopyrum tataricum* transgenic HRs produced higher levels of phenolic compounds, such as caffeic acid, chlorogenic acid, catechin, gallic acid, ferulic acid, quercetin, and rutin. In another study, the chlorogenic acid, protocatechuic acid, and ferulic acid contents were higher in the transgenic HRs of tomatoes [39]. In addition, the glucosinolates and phenolic compounds such as 4-methoxyglucobrassicin, 4-hydroxyglucobrassicin, glucoallysin, glucobrassicanapin, glucobrassicin, gluconapin, gluconasturtiin, neoglucobrassicin, progoitrin, sinigrin, catechin, ferulic acid, cinnamic acid, chlorogenic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, quercetin, and rutin were high in the transgenic HRs of *Brassica rapa* spp. *rapa* [32]. In this study, we identified most of the glucosinolate and phenolic compounds in the green and red kimchi cabbage HRs. Therefore, most of the HRs shared common glucosinolates and phenolic compounds.

Light plays a vital role in the growth, biosynthesis, and production of SMs, such as anthocyanins, flavonoids, and terpenoids [40–45]. The stimulatory effects of light on SM production have been reported in several plants, such as *Artimisia annua* [46], *O. basilicum* [31], *Perilla frutiescnens* [47], *Peteroselinum hortense*, and *Rudbeckia hirata* [48]. In contrast, light also has an inhibitory effect on SM accumulation, such as for shikonin and nicotine [49]. In this study, most of the individual glucosinolate and phenolic metabolites were significantly higher in the dark and light conditions, respectively. The accumulation of rosmarinic acid content varied in *O. basilicum* HRs when cultured under light and dark conditions [31]. In a previous study, the amino acid level of *B. juncea* increased when grown under dark conditions [50]. In contrast, higher anthocyanin accumulation was achieved in Tartary buckwheat T10 sprouts grown under light conditions compared to those grown under dark conditions [50]. In another study, the accumulation of compounds varied based on light or dark conditions. For example, free amino acids and γ-aminobutyric acid were enhanced in *B. juncea* seedlings when they were grown under light and dark conditions, respectively [51]. These studies have shown that a specific condition might be efficient for the induction of specific compounds in a particular species.

Different cultivars (Tartary buckwheat Hokkai T8 and T10) grown under similar conditions showed differential phenolic content accumulation [52]. This is consistent with our study results because we observed that the green and red kimchi cultivars showed varied production and accumulation of glucosinolate and phenolic contents in the HRs of kimchi cabbage. In addition, neoglucobrassicin, followed by 4-methoxyglucobrassicin and glucobrassicin, comprised the highest portion of the glucosinolates in the HRs of green and red kimchi. A similar result was obtained in green and red kale HRs, where the highest to lowest glucosinolate contents occurred in the following order: neoglucobrassicin, 4-methoxyglucobrassicin, and glucobrassicin [53]. In addition, the HRs of broccoli cultured in MS medium showed the highest accumulation of neoglucobrassicin content [54]. In this study, the neoglucobrassicin, 4-methoxyglucobrassicin, and glucobrassicin contents were significantly higher in green kimchi HRs, red kimchi HRs, and red kimchi HRs, respectively. In contrast to this study, in the HRs of green kale, the neoglucobrassicin accumulation was lowest, whereas the 4-methoxyglucobrassicin and glucobrassicin accumulation were significantly higher when cultured using MS medium [55]. Therefore, most HRs consist of common glucosinolates. However, the levels might differ based on the species.

5. Conclusions

HR cultures are an important alternative strategy for the mass production of valuable health-promoting SMs, such as glucosinolates and phenolic compounds, in kimchi cabbage. The HR growth in the GKHD condition showed the highest DW compared to that of the light conditions. HRs produced more glucosinolates in the GKHD condition, whereas the highest phenolic compound content was achieved in the RKHL condition. These findings shed light on the underlying mechanisms of the abiotic elicitors that enhance glucosinolate and phenolic compounds in kimchi cabbage HRs and can potentially be used as "biological factories" for the large-scale production of bioactive substances, such as glucosinolates and phenolic compounds. However, the antioxidant activity of HRs

is currently under examination. In the future, further studies will be required to fully comprehend the influences of various light intensities and light quality on glucosinolate and phenylpropanoid pathway gene expression and the accumulation of associated compounds in the HR cultures of different kimchi cabbage cultivars.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae9040466/s1, Figure S1: Individual metabolites that are significantly ($p \leq 0.05$) higher in hairy roots of green and red kimchi cabbage grown under light and dark conditions; Table S1: HPLC conditions for phytochemical analysis of metabolites.

Author Contributions: Conceptualization, S.Y.L., R.S. and S.U.P.; methodology, H.K., J.K.K., C.H.P. and R.S.; software, R.S.; validation, S.Y.L., R.S. and S.U.P.; formal analysis, H.K., J.K.K., C.H.P. and R.S.; investigation, S.Y.L. and S.U.P.; resources, S.Y.L. and S.U.P.; data curation, H.K., J.K.K., C.H.P. and R.S.; writing—original draft preparation, S.Y.L. and R.S.; writing—review and editing, S.Y.L., R.S. and S.U.P.; visualization, S.Y.L. and S.U.P.; supervision, S.Y.L. and S.U.P.; project administration, S.Y.L. and S.U.P.; funding acquisition, S.U.P. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by a research fund from Chosun University in 2022.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data reported are available in the Supplementary Materials.

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

References

1. Warwick, S.I. Brassicaceae in agriculture. In *Genetics and Genomics of the Brassicaceae*; Schmidt, R., Bancroft, I., Eds.; Springer: New York, NY, USA, 2011; pp. 33–65, ISBN 978-1-4419-7118-0.
2. Li, Z.; Lee, H.W.; Liang, X.; Liang, D.; Wang, Q.; Huang, D.; Ong, C.N. Profiling of phenolic compounds and antioxidant activity of 12 cruciferous vegetables. *Molecules* **2018**, *23*, 1139. [CrossRef] [PubMed]
3. Seong, G.-U.; Hwang, I.-W.; Chung, S.-K. Antioxidant capacities and polyphenolics of Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*) leaves. *Food Chem.* **2016**, *199*, 612–618. [CrossRef] [PubMed]
4. Kim, J.K.; Chu, S.M.; Kim, S.J.; Lee, D.J.; Lee, S.Y.; Lim, S.H.; Ha, S.-H.; Kweon, S.J.; Cho, H.S. Variation of glucosinolates in vegetable crops of *Brassica rapa* L. ssp. *pekinensis*. *Food Chem.* **2010**, *119*, 423–428. [CrossRef]
5. Lee, M.-K.; Chun, J.-H.; Byeon, D.H.; Chung, S.-O.; Park, S.U.; Park, S.; Arasu, M.V.; Al-Dhabi, N.A.; Lim, Y.-P.; Kim, S.-J. Variation of glucosinolates in 62 varieties of Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*) and their antioxidant activity. *LWT-Food Sci. Technol.* **2014**, *58*, 93–101. [CrossRef]
6. Xie, L.; Li, F.; Zhang, S.; Zhang, H.; Qian, W.; Li, P.; Zhang, S.; Sun, R. Mining for candidate genes in an introgression line by using RNA sequencing: The anthocyanin overaccumulation phenotype in Brassica. *Front. Plant Sci.* **2016**, *7*, 1245. [CrossRef]
7. Lee, H.; Oh, I.-N.; Kim, J.; Jung, D.; Cuong, N.P.; Kim, Y.; Lee, J.; Kwon, O.; Park, S.U.; Lim, Y. Phenolic compound profiles and their seasonal variations in new red-phenotype head-forming Chinese cabbages. *LWT* **2018**, *90*, 433–439. [CrossRef]
8. Sønderby, I.E.; Geu-Flores, F.; Halkier, B.A. Biosynthesis of glucosinolates–gene discovery and beyond. *Trends Plant Sci.* **2010**, *15*, 283–290. [CrossRef]
9. Clarke, D.B. Glucosinolates, structures and analysis in food. *Anal. Methods* **2010**, *2*, 310–325. [CrossRef]
10. Sathasivam, R.; Park, S.U.; Kim, J.K.; Park, Y.J.; Kim, M.C.; Nguyen, B.V.; Lee, S.Y. Metabolic profiling of primary and secondary metabolites in kohlrabi (*Brassica oleracea* var. *gongylodes*) sprouts exposed to different light-emitting diodes. *Plants* **2023**, *12*, 1296. [CrossRef]
11. Fernandez-Panchon, M.; Villano, D.; Troncoso, A.; Garcia-Parrilla, M. Antioxidant activity of phenolic compounds: From in vitro results to in vivo evidence. *Crit. Rev. Food Sci. Nutr.* **2008**, *48*, 649–671. [CrossRef]
12. Crozier, A.; Jaganath, I.B.; Clifford, M.N. Phenols, polyphenols and tannins: An overview. *Plant Second. Metab. Occur. Struct. Role Hum. Diet* **2006**, *1*, 1–25.
13. Ambriz-Pérez, D.L.; Leyva-López, N.; Gutierrez-Grijalva, E.P.; Heredia, J.B. Phenolic compounds: Natural alternative in inflammation treatment. A Review. *Cogent Food Agric.* **2016**, *2*, 1131412.
14. Cushnie, T.T.; Lamb, A.J. Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents* **2005**, *26*, 343–356. [CrossRef] [PubMed]
15. Rice-Evans, C.; Miller, N.; Paganga, G. Antioxidant properties of phenolic compounds. *Trends Plant Sci.* **1997**, *2*, 152–159. [CrossRef]
16. Mauro, M.L.; Bettini, P.P. *Agrobacterium rhizogenes* rolB oncogene: An intriguing player for many roles. *Plant Physiol. Biochem.* **2021**, *165*, 10–18. [CrossRef]

17. Kim, Y.-K.; Sathasivam, R.; Kim, Y.B.; Kim, J.K.; Park, S.U. Transcriptomic analysis, cloning, characterization, and expression analysis of triterpene biosynthetic genes and triterpene accumulation in the hairy roots of *Platycodon grandiflorum* exposed to methyl jasmonate. *ACS Omega* **2021**, *6*, 12820–12830. [CrossRef]
18. Morey, K.J.; Peebles, C.A. Hairy roots: An untapped potential for production of plant products. *Front. Plant Sci.* **2022**, *13*, 937095. [CrossRef]
19. Roy, A. Hairy root culture an alternative for bioactive compound production from medicinal plants. *Curr. Pharm. Biotechnol.* **2021**, *22*, 136–149. [CrossRef]
20. Kim, Y.; Wyslouzil, B.E.; Weathers, P.J. Secondary metabolism of hairy root cultures in bioreactors. *Vitr. Cell. Dev. Biol.-Plant* **2002**, *38*, 1–10. [CrossRef]
21. Hussain, M.J.; Abbas, Y.; Nazli, N.; Fatima, S.; Drouet, S.; Hano, C.; Abbasi, B.H. Root cultures, a boon for the production of valuable compounds: A comparative review. *Plants* **2022**, *11*, 439. [CrossRef] [PubMed]
22. Abbasi, B.H.; Tian, C.-L.; Murch, S.J.; Saxena, P.K.; Liu, C.-Z. Light-enhanced caffeic acid derivatives biosynthesis in hairy root cultures of *Echinacea purpurea*. *Plant Cell Rep.* **2007**, *26*, 1367–1372. [CrossRef]
23. Mukherjee, C.; Samanta, T.; Mitra, A. Redirection of metabolite biosynthesis from hydroxybenzoates to volatile terpenoids in green hairy roots of *Daucus carota*. *Planta* **2016**, *243*, 305–320. [CrossRef]
24. Yu, K.-W.; Murthy, H.N.; Hahn, E.-J.; Paek, K.-Y. Ginsenoside production by hairy root cultures of *Panax ginseng*: Influence of temperature and light quality. *Biochem. Eng. J.* **2005**, *23*, 53–56. [CrossRef]
25. Murashige, T.; Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant* **1962**, *15*, 473–497. [CrossRef]
26. Sathasivam, R.; Choi, M.; Radhakrishnan, R.; Kwon, H.; Yoon, J.; Yang, S.H.; Kim, J.K.; Chung, Y.-S.; Park, S.U. Effects of various strains of *Agrobacterium rhizogenes* strains on hairy root induction and analyses of primary and secondary metabolites in *Ocimum basilicum*. *Front. Plant Sci.* **2022**, *13*, 983776. [CrossRef]
27. Park, C.H.; Park, Y.E.; Yeo, H.J.; Park, N.I.; Park, S.U. Effect of light and dark on the phenolic compound accumulation in Tartary buckwheat hairy roots overexpressing *ZmLC*. *Int. J. Mol. Sci.* **2021**, *22*, 4702. [CrossRef] [PubMed]
28. Sathasivam, R.; Kim, M.C.; Yeo, H.J.; Nguyen, B.V.; Sohn, S.I.; Park, S.U.; Kim, J. Accumulation of phenolic compounds and glucosinolates in sprouts of pale green and purple kohlrabi (*Brassica oleracea* var. *gongylodes*) under light and dark conditions. *Agronomy* **2021**, *11*, 1939. [CrossRef]
29. Choi, M.; Sathasivam, R.; Nguyen, B.V.; Park, N.I.; Woo, S.-H.; Park, S.U. Expression analysis of phenylpropanoid pathway genes and metabolomic analysis of phenylpropanoid compounds in adventitious, hairy, and seedling roots of Tartary buckwheat. *Plants* **2021**, *11*, 90. [CrossRef] [PubMed]
30. Su, W.W.; Lee, K.-T. Plant cell and hairy root cultures–Process characteristics, products, and applications. In *Bioprocessing for Value-Added Products from Renewable Resources*; Su, W.W., Lee, K.-T., Eds.; Elsevier: Amsterdam, The Netherlands, 2007; pp. 263–292.
31. Kwon, D.Y.; Kim, Y.B.; Kim, J.K.; Park, S.U. Production of rosmarinic acid and correlated gene expression in hairy root cultures of green and purple basil (*Ocimum basilicum* L.). *Prep. Biochem. Biotechnol.* **2021**, *51*, 35–43. [CrossRef] [PubMed]
32. Chung, I.-M.; Rekha, K.; Rajakumar, G.; Thiruvengadam, M. Production of glucosinolates, phenolic compounds and associated gene expression profiles of hairy root cultures in turnip (*Brassica rapa* ssp. *rapa*). *3 Biotech* **2016**, *6*, 1–16. [CrossRef] [PubMed]
33. Weremczuk-Jeżyna, I.; Grzegorczyk-Karolak, I.; Frydrych, B.; Królicka, A.; Wysokińska, H. Hairy roots of *Dracocephalum moldavica*: Rosmarinic acid content and antioxidant potential. *Acta Physiol. Plant* **2013**, *35*, 2095–2103. [CrossRef]
34. Thiruvengadam, M.; Praveen, N.; Maria John, K.; Yang, Y.-S.; Kim, S.-H.; Chung, I.-M. Establishment of *Momordica charantia* hairy root cultures for the production of phenolic compounds and determination of their biological activities. *Plant Cell Tiss. Org. Cult.* **2014**, *118*, 545–557. [CrossRef]
35. Georgiev, V.G.; Weber, J.; Kneschke, E.-M.; Denev, P.N.; Bley, T.; Pavlov, A.I. Antioxidant activity and phenolic content of betalain extracts from intact plants and hairy root cultures of the red beetroot *Beta vulgaris* cv. Detroit dark red. *Plant Foods Hum. Nutr.* **2010**, *65*, 105–111. [CrossRef] [PubMed]
36. Bhandari, S.R.; Jo, J.S.; Lee, J.G. Comparison of glucosinolate profiles in different tissues of nine Brassica crops. *Molecules* **2015**, *20*, 15827–15841. [CrossRef] [PubMed]
37. Park, N.I.; Kim, J.K.; Park, W.T.; Cho, J.W.; Lim, Y.P.; Park, S.U. An efficient protocol for genetic transformation of watercress (*Nasturtium officinale*) using *Agrobacterium rhizogenes*. *Mol. Biol. Rep.* **2011**, *38*, 4947–4953. [CrossRef]
38. Kim, Y.K.; Li, X.; Xu, H.; Il Park, N.; Uddin, M.R.; Pyon, J.Y.; Park, S.U. Production of phenolic compounds in hairy root culture of tartary buckwheat (*Fagopyrum tataricum* Gaertn). *J. Crop Sci. Biotechnol.* **2009**, *12*, 53–57. [CrossRef]
39. Singh, H.; Dixit, S.; Verma, P.C.; Singh, P.K. Evaluation of total phenolic compounds and insecticidal and antioxidant activities of tomato hairy root extract. *J. Agric. Food Chem.* **2014**, *62*, 2588–2594. [CrossRef]
40. Gerhardt, K.E.; Lampi, M.A.; Greenberg, B.M. The effects of far-red light on plant growth and flavonoid accumulation in Brassica napus in the presence of ultraviolet B radiation. *Photochem. Photobiol.* **2008**, *84*, 1445–1454. [CrossRef]
41. Kawoosa, T.; Singh, H.; Kumar, A.; Sharma, S.K.; Devi, K.; Dutt, S.; Vats, S.K.; Sharma, M.; Ahuja, P.S.; Kumar, S. Light and temperature regulated terpene biosynthesis: Hepatoprotective monoterpene picroside accumulation in *Picrorhiza kurrooa*. *Funct. Integr. Genom.* **2010**, *10*, 393–404. [CrossRef]

42. Koyama, K.; Ikeda, H.; Poudel, P.R.; Goto-Yamamoto, N. Light quality affects flavonoid biosynthesis in young berries of Cabernet Sauvignon grape. *Phytochemistry* **2012**, *78*, 54–64. [CrossRef] [PubMed]
43. Lei, H.; Dong, B.-C.; Yang, X.-J.; Huang, C.-B.; Wang, X.-D.; Wu, X.-J. Effect of light on flavonoids biosynthesis in red rice Rdh. *Agric. Sci. China* **2009**, *8*, 746–752.
44. Vogt, T.; Ibdah, M.; Schmidt, J.; Wray, V.; Nimtz, M.; Strack, D. Light-induced betacyanin and flavonol accumulation in bladder cells of *Mesembryanthemum crystallinum*. *Phytochemistry* **1999**, *52*, 583–592. [CrossRef] [PubMed]
45. Wang, Y.; Gao, L.; Shan, Y.; Liu, Y.; Tian, Y.; Xia, T. Influence of shade on flavonoid biosynthesis in tea (*Camellia sinensis* (L.) O. Kuntze). *Sci. Hortic.-Amst.* **2012**, *141*, 7–16. [CrossRef]
46. Liu, C.-Z.; Guo, C.; Wang, Y.-C.; Ouyang, F. Effect of light irradiation on hairy root growth and artemisinin biosynthesis of *Artemisia annua* L. *Process Biochem.* **2002**, *38*, 581–585. [CrossRef]
47. Zhong, J.J.; Seki, T.; Kinoshita, S.I.; Yoshida, T. Effect of light irradiation on anthocyanin production by suspended culture of *Perilla frutescens*. *Biotechnol. Bioeng.* **1991**, *38*, 653–658. [CrossRef]
48. Łuczkiewicz, M.; Zárate, R.; Dembińska-Migas, W.; Migas, P.; Verpoorte, R. Production of pulchelin E in hairy roots, callus and suspension cultures of *Rudbeckia hirta* L. *Plant Sci.* **2002**, *163*, 91–100. [CrossRef]
49. Tabata, M.; Mizukami, H.; Hiraoka, N.; Konoshima, M. Pigment formation in callus cultures of *Lithospermum erythrorhizon*. *Phytochemistry* **1974**, *13*, 927–932. [CrossRef]
50. Park, N.I.; Li, X.; Suzuki, T.; Kim, S.-J.; Woo, S.-H.; Park, C.H.; Park, S.U. Differential expression of anthocyanin biosynthetic genes and anthocyanin accumulation in tartary buckwheat cultivars 'Hokkai T8'and 'Hokkai T10'. *J. Agric. Food Chem.* **2011**, *59*, 2356–2361. [CrossRef]
51. Li, X.; Kim, Y.B.; Uddin, M.R.; Lee, S.; Kim, S.-J.; Park, S.U. Influence of light on the free amino acid content and γ-aminobutyric acid synthesis in *Brassica juncea* seedlings. *J. Agric. Food Chem.* **2013**, *61*, 8624–8631. [CrossRef]
52. Thwe, A.A.; Kim, J.K.; Li, X.; Bok Kim, Y.; Romij Uddin, M.; Kim, S.J.; Suzuki, T.; Park, N.I.; Park, S.U. Metabolomic analysis and phenylpropanoid biosynthesis in hairy root culture of tartary buckwheat cultivars. *PLoS ONE* **2013**, *8*, e65349. [CrossRef]
53. Cuong, D.M.; Park, S.U.; Park, C.H.; Kim, N.S.; Bong, S.J.; Lee, S.Y. Comparative analysis of glucosinolate production in hairy roots of green and red kale (*Brassica oleracea* var. *acephala*). *Prep. Biochem. Biotechnol.* **2019**, *49*, 775–782. [CrossRef] [PubMed]
54. Kim, H.H.; Kwon, D.Y.; Bae, H.; Kim, S.-J.; Kim, Y.B.; Uddin, M.R.; Park, S.U. Influence of auxins on glucosinolate biosynthesis in hairy root cultures of broccoli (*Brassica oleracea* var. *italica*). *Asian J. Chem.* **2013**, *25*, 6099. [CrossRef]
55. Lee, S.Y.; Bong, S.J.; Kim, J.K.; Park, S.U. Glucosinolate biosynthesis as influenced by growth media and auxin in hairy root cultures of kale (*Brassica oleracea* var. *acephala*). *Emir. J. Food Agric.* **2016**, 277–282. [CrossRef]