Journal of Bio-Environment Control, Vol. 31, No. 4:332-342, October (2022)
DOI https://doi.org/10.12791/KSBEC.2022.31.4.332
pISSN 1229-4675
eISSN 2765-3641

Glucosinolate Content Varies and Transcriptome Analysis in Different Kale Cultivars (*Brassica oleracea* var. *acephala*) Grown in a Vertical Farm

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Abstract. Kale (*Brassica oleracea* var. *acephala*) is one of the most frequently consumed leafy vegetables globally, as it contains numerous nutrients; essential amino acids, phenolics, vitamins, and minerals, and is particularly rich in glucosinolates. However, the differences in the biosynthesis of glucosinolates and related gene expression among kale cultivars has been poorly reported. In this study, we investigated glucosinolates profile and content in three different kale cultivars, including green ('Man-Choo' and 'Mat-Jjang') and red kale ('Red-Curled') cultivars grown in a vertical farm, using transcriptomic and metabolomic analyses. The growth and development of the green kale cultivars were higher than those of the red kale cultivar at 6 weeks after cultivation. High-performance liquid chromatography (HPLC) analysis revealed five glucosinolates in the 'Man-Choo' cultivar, and four glucosinolates in the 'Mat-Jjang' and 'Red-Curled' cultivars. Glucobrassicin was the most predominant glucosinolate followed by gluconastrutiin in all the cultivars. In contrast, other glucosinolates were highly dependent to the genotypes. The highest total glucosinolates was found in the 'Red-Curled' cultivar, which followed by 'Man-Choo' and 'Mat-Jjang'. Based on transcriptome analysis, eight genes were involved in glucosinolate biosynthesis. The overall results suggest that the glucosinolate content and accumulation patterns differ according to the kale cultivar and differential expression of glucosinolate biosynthetic genes.

Additional key words: glucosinolates, growth, kale, metabolome, transcriptome, vertical farms

Introduction

The Brassicaceae family (cruciferous plant family) contains important vegetables that are widely consumed due to their health-promoting properties, as they contain various nutrients and phytochemicals, such as glucosinolates, phenolic compounds, anthocyanins, carotenoids, and vitamins (Jahangir et al., 2009; Lee et al., 2015). These phytochemicals induce detoxification enzyme activation, scavenge free radicals, alleviate inflammation, stimulate the immune system, inhibit malignant transformation, and regulate the growth of cancer cells (Mayne, 1996). Kale (*Brassica oleracea* var. *acephala*), a member of the Brassicaceae family, is widely cultivated in Asia, Europe, and the United States of America. Kale contains a wide range of phytochemicals and is particularly rich in glucosinolates (Ayaz et al., 2006; Lännenpää, 2014).

Glucosinolates and their derivatives assist plants in defense against pathogens and insects; they also exhibit a wide range of pharmaceutical and biological activities associated with a reduced risk of breast, liver, lung, stomach, pancreas, colon, rectum, prostate, and colorectal cancers in humans (Herr and Büchler, 2010). Glucosinolates are divided into three groups: aliphatic, indolic and aromatic glucosinolates (Sønderby et al., 2010; Yan and Chen, 2007).

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Received July 22, 2022; Revised October 11, 2022; Accepted October 14, 2022

Glucosinolate biosynthesis occurs in three independent phases: side chain elongation of precursor amino acids with an additional methylene group, partial amino acid conversion to form the core structure, and secondary modification of the amino acid side chain (Sønderby et al., 2010).

The content and composition of glucosinolates and phenolic compounds in kale varies depending on the cultivation facility (open field, greenhouse, and vertical farms), environmental conditions (temperature, relative humidity, light intensity, and light quality), and carbon dioxide (CO_2) levels (Lee et al., 2016; Neugart et al., 2018). Kale can be easily cultivated in open field using conventional methods; however, the yield and content of glucosinolates and phenolic compounds are not stable and high throughout the year owing to the extremely fluctuating climate conditions and soil-borne diseases (Kim and Chung, 2018), which leads to unsustainable production and unstable prices in the market. Controlled-environment agriculture systems (greenhouses and vertical farms) have been developed to overcome the abovementioned challenges, and kale production has been shifted to these systems to improve plant growth and quality. Controlled-environment agriculture systems control internal environmental conditions, provide multiple-shelf cultivation, reduce resource use (water and fertilizer) and labor cost, and help in preventing disease and pest infestation, leading to fast and sustainable yield, and the abundance of phytochemicals in plants (Kozai, 2013). Kale plants grown in vertical farms have higher glucosinolate content than plants grown in greenhouses and open fields (Kim and Chung, 2018).

Recently, advances in omics approaches have offered new strategies to explore the biosynthetic pathways of secondary metabolites and their molecular roles in plants. Transcriptome analysis can provide a fundamental key to the interpretation of gene function by using regulatory sequence analysis, gene ontology, and pathway information. In this study, we explored the secondary metabolite biosynthesis pathway and its molecular role in three different kale cultivars. Overall, the data obtained from transcriptomic and metabolomic analyses will be helpful for selecting cultivars in vertical farms with respect to glucosinolate production and in investigating gene-to-metabolite connections in kale.

Materials and Methods

1. Plant materials

Three kale cultivars, including green kale (‘Man-Choo Collard’ and ‘Mat-Jjang’) and red kale (‘Red-Curled’), were used as the plant materials in this study.

2. Growth conditions

Seeds of the three cultivars (Asia Seed, Seoul, Korea) were sown in a petri dish, irrigated with distilled water, and grown at 20 ± 5°C under a fluorescent lamp, with a photosynthetic photon flux density (PPFD) of 120 μmol·m^{-2}·s^{-1} and a photoperiod of 12 h. Two days after sowing, the seedlings were transplanted on a cultivation panel (25 × 17.5 × 3 cm, L × W × H) and irrigated with distilled water. Seven days after sowing, Hoagland nutrient solution was filled in a plastic container (45 × 28 × 12 cm, L × W × H), and added to three trays (for each cultivar) in a cultivation panel (41 × 23.5 × 2.5 cm, L × W × H). A total of 45 plants per cultivar were cultivated in a deep flow technique system under the following conditions: temperature, 20 ± 5°C; PPDF, 140 μ mol·m^{-2}·s^{-1}; and photoperiod, 12 h. Hoagland nutrient solution (1 L; pH of 6.0, electrical conductivity of 1.0 dS·m^{-1}) was supplied once every 3 days.

The plants were harvested for growth measurements at six weeks after sowing. The shoots and roots of each plant were separated, and fresh weight was measured using an electronic scale (PAG214C, Ohaus Corporation, NJ, USA). The shoots and roots were dried in an oven (WOF-155, DAIHAN, Korea) at 70°C for 72 h, and their dry weights were measured. The leaf area was measured using an area meter (LI-3100C, Li-Cor, Lincoln, NE, USA).

3. Glucosinolate analysis

The samples were prepared for glucosinolate analysis using the method described by Jo et al. (2018). The freeze-dried powder samples (50 mg) and 70% methanol (1 mL) were mixed in a 2 mL screw-cap tube, extracted in a water bath at 75°C for 1 h, and centrifuged at 10,000 × g at 4°C for 20 min. After centrifugation, the supernatant was collected in a 2 mL tube and the pellet was subjected to another round of extraction using the same procedure. The collected

supernatant was loaded onto a mini-biospin chromatography column filled with 0.7 mL DEAE-Sephadex A25 (Sigma-Aldrich, St. Louis, MO, USA) slurry for desulfation. This slurry was pre-activated with 0.1 M sodium acetate pH 4.0. (Sigma-Aldrich, St. Louis, MO, USA). The desulfurization of intact glucosinolate was performed using 200 μL of aryl sulfatase (EC 3.1.6.1, H-1 type from *Helix pomatia*; Sigma-Aldrich, St. Louis, MO, USA). The column was desulfurized at room temperature for 18 h, eluted three times with 0.5 mL of HPLC grade distilled water, filtered through a 0.2 μm polytetrafluoroethylene syringe filter, and stored in 1.5 mL vials. The samples (10 μL) were analyzed using an Agilent 1260 HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a diode array detector (DAD) set at 229, using an Acquity UPLC BEH C18 column (1.7 μm, 2.1 × 100 mm; Waters Co., Milford, MA, USA); individual glucosinolates (GSLs) were separated by gradient elution of solvent A (100% distilled water) and solvent B (20% acetonitrile in water) at a flow rate of 0.2 $mL \cdot min^{-1}$. The gradient program was as follows: a linear step from 1 to 30% solvent B within 6 min, 30 − 50% solvent B within 10 min, 50 − 99% solvent B for 11 min, followed by a constant condition for up to 14 min, a rapid decline to 1% solvent B for up to 15 min, and an isocratic flow of 1% solvent B for up to 25 min. A pure commercial glucosinolate standard was desulfurized, using the same pretreatment process as the sample, and used for the identification and quantification of GSL peaks ($\mu mol \cdot g^{-1}$).

4. RNA Preparation

The third leaves of the three kale cultivars were freeze-dried and crushed using an Automill machine (TK-AM7, Tokken Inc., Kashiwa, Japan) for 2 min for RNA-Seq analysis and quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Total RNA was isolated using a RNeasy Plant Mini Kit (Qiagen N.V., Venlo, The Netherlands) following the manufacturer's instructions. RNA quantity and purity were determined by evaluating the absorbance at 260 nm and 260/280 nm absorbance ratio, respectively, using a spectrophotometer (NanoDrop Lite, Thermo Fisher Scientific, Waltham, MA, USA).

5. RNA-Seq analysis

Raw data were extracted using the software provided by Agilent Feature Extraction Software (v11.0.1.1). The raw data for the same gene were summarized automatically using the Agilent feature extraction protocol to generate a raw data text file, providing expression data for each gene probed on the array. Array probes with FLAG A in the samples were filtered out. The selected gProcessedSignal value was logarithmically transformed and normalized using the quantile method. The statistical significance of the expression data was determined using fold change. Hierarchical cluster analysis was performed using complete linkage and Euclidean distance as similarity measures. Gene enrichment and functional annotation analysis for the significant probe list was performed using Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses (http://kegg.jp). All data analysis and visualization of differentially expressed genes were conducted using R 3.0.2 (www.r-project.org).

6. Statistical analysis

All recorded parameters were collected and obtained from five harvested plants (n = 5). Analysis of variance was performed for statistical analysis, using the SAS 9.2 program (SAS Institute Inc., Cary, NC, USA) with, and one-way ANOVA followed by Tukey's test was used to derive the significant differences for all treatments verified with $p < 0.05$.

Results

1. Growth characteristics

The three kale cultivars grown under controlled environmental conditions showed significant differences in growth response (Fig. 1). The shoot fresh and dry weights of both the green kale cultivars were higher than those of the red kale cultivar (Fig. 1A, 1B). In particular, shoot fresh and dry weights of 'Man-Choo' were 1.4- and 1.7-fold higher than those of 'Red-Curled' plants, respectively. The leaf area showed a similar trend to that of the shoots, with a higher leaf area (1.6 times) in 'Man-Choo' than in 'Red-Curled' plants (Fig. 1C). Non-significant differences were observed

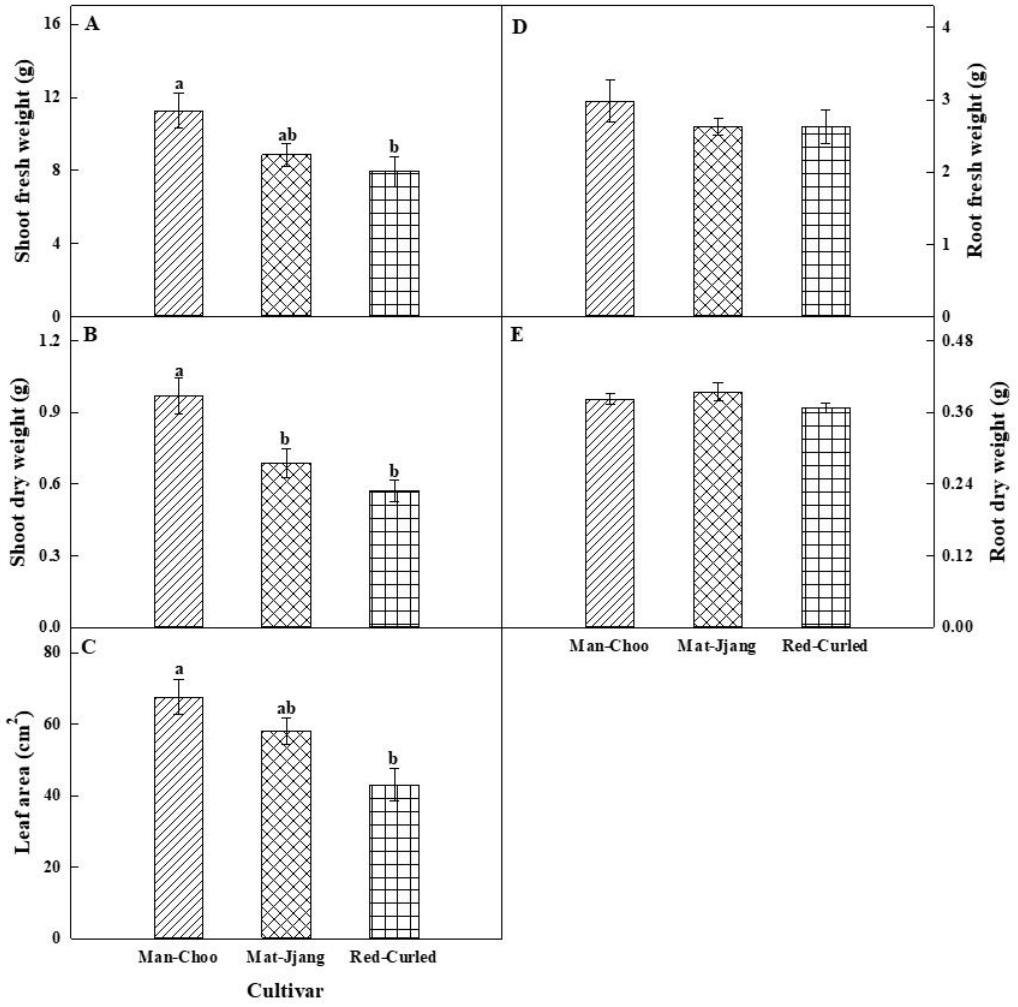

Fig. 1. Shoot fresh (A) and dry (B) weights, leaf area (C), and root fresh (D) and dry (E) weights of three kale cultivars, namely 'Man-Choo', 'Mat-Jjang', and 'Red-Curled', grown in a vertical farm for 6 weeks. Different letters within a figure indicates statistically significant using Tukey's test at $p > 0.05$.

in the root fresh and dry weights among the three cultivars (Fig. 1D, 1E).

2. Quantification of individual glucosinolate content in the three kale cultivars

The content of individual glucosinolates in the three kale cultivars was measured, and seven individual glucosinolates were detected in some of the three kale cultivars (Fig. 2A). Glucoiberin (IBR) and sinigrin (SIN) were only identified in 'Man-Choo' and 'Mat-Jjang' plants, respectively. Gluconapin (NAP) was detected in 'Man-Choo' and 'Mat-Jjang' plants. Progoitrin (PRO), glucoraphanin (GRA), glucobrassicin (BRA), and gluconasturtiin (NAS) were detected in all three cultivars, with BRA and NAS accounting for the highest content among individual glucosinolates. The content of PRO in 'Red-Curled' plants was approximately 7.3- and 6.9-fold higher than in 'Man-Choo' and 'Mat-Jjang' plants, respectively. 'Red-Curled' plants also had 2.6- and 4.03-fold higher NAS content than 'Man-Choo' and 'Mat-Jjang' plants, respectively. The contents of GRA and BRA were the highest in 'Man-Choo' plants, whereas the contents of individual glucosinolates were relatively low in 'Mat-Jjang' plants. Significantly ($p < 0.05$) higher total glucosinolate content was observed in 'Man-Choo' and 'Red-Curled' plants than in 'Mat-Jjang' plants (Fig. 2B).

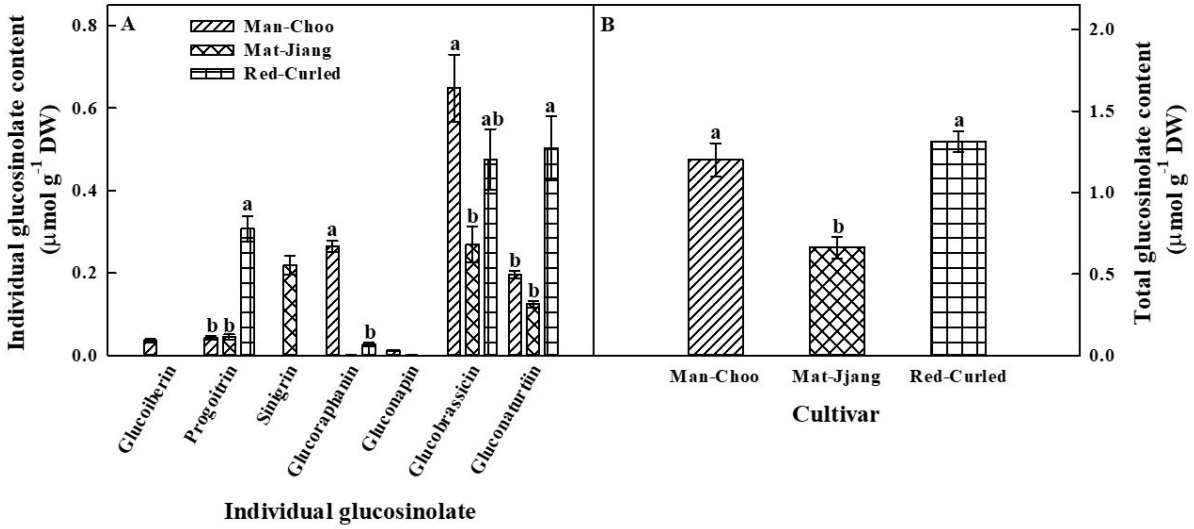

Fig. 2. Individual (A) and total (B) glucosinolate contents of the three kale cultivars, namely 'Man-Choo', 'Mat-Jjang', and 'Red-Curled', grown in a vertical farm for 6 weeks. Different letters within a figure indicates statistically significant using Tukey's test at $p > 0.05$.

Table 1. Summary of the sequence assembly after Illumina sequencing of the three kale cultivars.

Cultivars	Read bases	Total reads	Mean length (bp)	GC (%)	Q20 (%)	Q30 (%)
			Raw data			
Man-Choo	7,023,986,824	69,544,424	101	51.25	98.78	96.31
Mat-Jjang	7,445,568,904	73,718,504	101	52.33	98.71	95.80
Red-Curled	6,498,195,974	64,338,574	101	50.46	98.77	96.25
			Trimmed data			
Man-Choo	6,912,272,036	68,637,586	100.71	51.27	99.19	96.87
Mat-Jjang	9,346,631,913	72,921,126	100.75	52.36	99.02	96.25
Red-Curled	6,399,554,129	63,559,766	100.69	50.48	99.17	96.80

3. Illumina sequencing and de novo transcriptome assembly of the three kale cultivars

RNA sequencing analysis was performed using total RNA extracted from the three kale cultivars (Table 1). The cDNA fragments of the three kale cultivars obtained through RNA-seq were mapped to a known genomic DNA reference genome (GCF_000695525.1_BOL) using the HISAT2 program, which can perform splice junction processing with coverages of 85% and 82%, respectively. Differentially expressed genes (DEGs) and the number of up- and down-regulated genes based on fold change of expression level for each comparative combination were defined (Fig. 3). A total of 3,087 DEGs with 1,529 upregulated genes

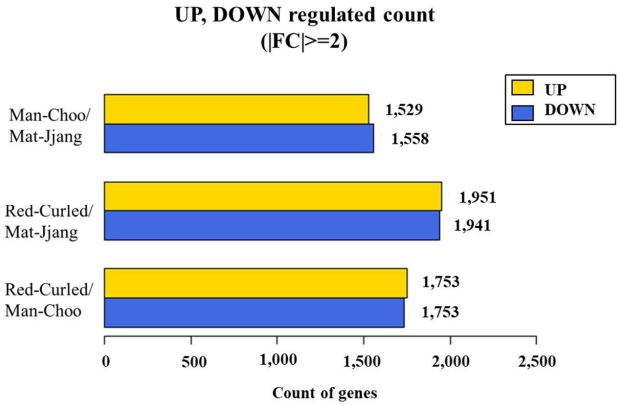

Fig. 3. Number of upregulated and downregulated genes based on the fold change of expression for the three kale cultivars.

were found when the transcriptome profile of 'Man-Choo' plants was compared to that of 'Mat-Jjang' plants; 3,892 DEGs with 1,951 upregulated genes were obtained when the transcriptome profile of 'Red-Curled' plants was compared to that of 'Mat-Jjang' plants; and 3,488 DEGs with 1,753 upregulated genes were obtained when the transcriptome profile of 'Red-Curled' plants was compared to that of 'Man-Choo' plants.

4. Identification of glucosinolate and phenylpropanoid synthesis genes from the transcriptomes of the three kale cultivars

Transcriptome analysis of kale cultivars revealed genes involved in the biosynthesis of secondary metabolites, including glucosinolates, phenolics, and anthocyanins (Table 2 and 3). Seven genes encoding enzymes related to the glucosinolate biosynthetic pathway from the transcriptome data of kale cultivars were identified, including isopropylmalate dehydratase (IPMDH), branched-chain-amino-acid amino-transferase 6 (BCAT6), cytochrome P450 79B1 (CYP79B1), glutathione S-transferase Z1 (GSTF), flavin-containing monooxygenase FMO GS-OX5-like (FMOGS-OX5), cytosolic sulfotransferase 16 (SOT16), and UDP-glycosyltransferase 72B3 (UGT72B2) (Table 2). The expression of most of these genes in 'Man-Choo' plants was decreased compared to that in 'Mat-Jjang' plants, except for the expression of FMOGS-OX5 and UGT72B2 genes, which increased by 2.09- and 3.93-fold, respectively. The expression of all genes in 'Red-Curled' plants was increased compared to that in 'Mat-Jjang' plants, and the expression of SOT16 and UGT72B2 genes displayed the highest increase, i.e., 2.76- and 2.46-fold, respectively. Comparative analysis of the transcriptomes of 'Red-Curled' and 'Man-Choo' plants revealed an increase in the expression of glucosinolate synthesis genes in 'Red-Curled' plants, with the SOT16 gene displaying the highest increase of 5.19-fold.

Five genes were related to the phenylpropanoid synthesis pathway, including phenylalanine ammonia-lyase 2-like (PAL2), chalcone synthase (CHS), 4-coumarate-CoA ligase 3 (4CL3), caffeic acid 3-O-methyltransferase-like (COMT),

Table 2. Differential expression of genes related to glucosinolate biosynthesis and phenylpropanoid in the three kale cultivars. Values indicate $\log_2$ ratios obtained based on the RNA-seq data.

Gene symbol	Entrez gene ID	Description	Fold change ($\log_2$ ratio)		
			Man-Choo / Mat-Jjang[z]	Red-Curled / Mat-Jjang	Red-Curled / Man-Choo
Glucosinolate transcript					
IPMDH	106342873	isopropylmalate dehydratase	–2.25	1.63	3.67
BCAT6	106296539	branched-chain-amino-acid aminotransferase 6	–1.03	1.96	2.01
CYP79B1	106302779	cytochrome P450 79B1	–1.58	1.78	2.81
GSTF	106307008	glutathione S-transferase Z1	–1.01	1.98	2.00
FMOGS-OX5	106312190	flavin-containing monooxygenase FMO GS-OX5-like	2.09	1.07	–1.95
SOT16	106327830	cytosolic sulfotransferase 16	–1.88	2.76	5.19
UGT72B2	106343816	UDP-glycosyltransferase 72B3	3.93	2.46	–1.60
Phenylpropanoid transcript					
PAL2	106319945	phenylalanine ammonia-lyase 2-like	–1.92	2.33	4.47
CHS	106327741	chalcone synthase	–2.43	–1.29	1.88
4CL3	106296384	4-coumarate--CoA ligase 3	1.18	2.06	1.73
COMT	106301103	caffeic acid 3-O-methyltransferase-like	1.61	2.39	1.49
DFR	106315758	dihydroflavonol-4-reductase	–3.43	–1.17	2.93

[z]Calculated based on cultivars.
Positive and negative values with increases of ≥2.0 or ≤2.0 are highlighted pink or green, respectively. To analyze reliable data, noise was excluded (signal evaluation).

and dihydroflavonol-4-reductase (DFR) genes. The expression levels of these genes were compared between each combination of two cultivars. The expression of PAL2, CHS, and DFR genes decreased in 'Man-Choo' plants compared to that in 'Mat-Jjang' plants, whereas the expression of 4CL3 and COMT genes was slightly increased. Comparative analysis of the transcriptomes of 'Red-Curled' and 'Mat-Jjang' plants revealed that the expression levels of PAL, 4CL3, and COMT genes were 2.33-, 2.06-, 2.39-fold higher than those in 'Mat-Jjang' plants. The expression of all identified phenylpropanoid synthesis genes was higher in 'Red-Curled' plants than in 'Man-Choo' plants.

Table 3. Upregulation or downregulation of genes related to the biosynthesis of secondary metabolites and glucosinolates in the three kale cultivars based on the KEGG database. Values indicate log_2 ratios obtained based on the RNA-seq data.

Entrez gene ID	Description (product)	Fold change (log_2 ratio)		
		Man-Choo / Mat-Jjang[z]	Red-Curled / Mat-Jjang	Red-Curled / Man-Choo
KEGG pathway in biosynthesis of secondary metabolites				
106296887	caffeic acid 3-O-methyltransferase-like	15.00	1.64	–9.13
106311835	bifunctional L-3-cyanoalanine synthase/cysteine synthase C1, mitochondrial	5.09	7.83	1.54
106300136	lipoxygenase 2, chloroplastic-like	–1.53	13.56	20.70
106311776	catalase-3-like	4.46	7.02	1.57
106335545	aspartate aminotransferase 3, chloroplastic	2.43	7.02	2.89
106301303	aldehyde dehydrogenase family 3 member F1-like	–2.04	6.54	13.32
106296631	diaminopimelate epimerase, chloroplastic	3.89	6.00	1.55
106294572	3-ketoacyl-CoA synthase 1	2.93	5.62	1.92
106307617	putative glucose-6-phosphate 1-epimerase	1.16	5.49	4.73
106334854	2-oxoglutarate dehydrogenase, mitochondrial	3.13	5.44	1.74
106295308	3-ketoacyl-CoA synthase 5	1.14	5.36	4.71
106335697	probable fructose-bisphosphate aldolase 2, chloroplastic	2.65	5.10	1.93
106312287	3-ketoacyl-CoA synthase 1	–1.46	4.96	7.23
106301496	probable tyrosine decarboxylase 2	–3.00	2.07	6.20
106295733	3-ketoacyl-CoA synthase 17	–1.22	4.96	6.04
106311920	phenylalanine ammonia-lyase 2	–2.44	2.29	5.59
106307643	tryptophan synthase alpha chain, chloroplastic	–1.68	3.02	5.08
106308419	protein ECERIFERUM 1	–1.22	4.09	5.01
KEGG pathway in glucosinolate biosynthesis				
106312180	cytochrome P450 83B1 (CYP 83A1)	–11.44	1.84	21.03
106327830	cytosolic sulfotransferase 16 (SOT16)	–1.88	2.76	5.19
106342873	3-isopropylmalate dehydratase large subunit	–2.25	1.63	3.67
106302779	cytochrome P450 79B1 (CYP 79B2)	–1.58	1.78	2.81
106296616	cytosolic sulfotransferase 16-like (ST5a)	1.35	3.78	2.79
106343089	dihomomethionine N-hydroxylase-like (CYP79F1)	–1.87	1.41	2.64
106306752	S-alkyl-thiohydroximate lyase SUR1 (SUR1)	1.20	3.12	2.60
106294846	UDP-glycosyltransferase 74B1 (UGT74B1)	1.84	2.47	1.34

[z]Calculated based on cultivars.

Differentially expressed genes were defined as genes with log2 fold change of ≤2 or ≥2 and an FDR (false discovery rate) of ≤0.01. Positive and negative values for increases of ≥2.0 or ≤2.0 are highlighted pink or green, respectively. To analyze reliable data, noise was excluded (signal evaluation).

5. KEGG pathway enrichment analyses of DEGs

Gene functions with an emphasis on the biological pathways were categorized using the KEGG pathway enrichment analyses. A total of 3,910 unigenes were assigned to 130 KEGG pathways. The largest number of unigenes was classified into metabolic pathways (boe01100, 774 genes, and 19.79%) and biosynthesis of secondary metabolites (boe01110, 365 genes, and 9.33%). Meanwhile, eight genes were involved in the glucosinolate synthesis pathway (boe00966, 0.2%), including cytochrome P450 83B1 (CYP 83A1), cytosolic sulfotransferase 16 (SOT16), 3-isopropylmalate dehydratase large subunit, cytochrome P450 79B1 (CYP 79B2), cytosolic sulfotransferase 16-like (ST5a), dihomomethionine N-hydroxylase-like (CYP79F1), S-alkyl-thiohydroximate lyase SUR1 (SUR1), and UDP-glycosyltransferase 74B1 (UGT74B1) genes. Most of these genes in 'Man-Choo' plants were downregulated compared to those in 'Mat-Jjang' plants, except for ST5, SUR1, and UGT74B1 genes. In contrast, these genes were upregulated in 'Red-Curled' plants compared to that in 'Mat-Jjang' and 'Man-Choo' plants. Similar results were obtained for some of the genes listed to be involved in the biosynthesis of secondary metabolites.

Discussion

In the present study, three kale cultivars grown under controlled environmental conditions exhibited an efficient increase in growth and development. However, the response of growth rate was slightly different among the three cultivars. Both green kale cultivars ('Man-Choo' and 'Mat-Jjang') showed higher shoot fresh and dry weights and leaf area than the 'Red-Curled' cultivar. These findings are in accordance with the results obtained for growth of different green and red cultivars of other species. A previous study also reported faster growth of green perilla cultivars than that of red perilla cultivars grown in walk-in type vertical farms, under the same electrical conditions of nutrient solution, light intensity, and light quality (Lu et al., 2017; Nguyen and Oh, 2021). The differences in growth between the cultivars could be closely related to their photosynthetic processes. Red leaf cultivars normally contain abundant anthocyanins, which can protect the photosynthetic apparatus from pho-toinhibition (Smillie and Hetherington, 1999). However, this property of anthocyanins can cause a reduction in the net photosynthetic rate of red leaf plants. Further, photosynthesis in red leaves is lower than that in green leaves (Burger and Edwards, 1996); which leads to lower growth of red-leaf vegetables than that of green-leaf vegetables.

Kale contains significantly higher amounts of indolic or aliphatic glucosinolates, although the ratio varies depending on the cultivar. In the present study, the content of glucobrassicin, an indolic glucosinolate, was found to be the highest among the individual glucosinolates in all three kale cultivars. Glucobrassicin is a common indolic glucosinolate that exhibits anti-breast cancer properties (Palani et al., 2016). Various aliphatic glucosinolates were detected in each of the three kale cultivars; however, glucoiberin and gluconapin were only found in 'Man-Choo' plants, sinigrin was only found in 'Mat-Jjang' plants, glucoraphanin was detected in 'Man-Choo' plants (highest content) and 'Red-Curled' plants (lowest content), and progoitrin had the highest content in 'Red-Curled' plants compared to that in 'Man-Choo' and 'Mat-Jjang' plants. These aliphatic glucosinolates have numerous therapeutic benefits, such as anticancer, antifungal, antioxidant, anti-inflammatory, and wound-healing properties (Awasthi and Saraswathi, 2016; Liu et al. 2012). Progoitrin was identified as the aliphatic glucosinolate with the highest ratio of abundance in red kale compared to that in green kale, while sinigrin was the most disproportionately abundant glucosinolate in green kale compared to that in red kale (Jeon et al., 2018). 'Red-Curled' cultivar showed lower growth and development than the green kale cultivars, but had a higher total glucosinolate content. Previous studies reported that red leaf kale contains higher levels of total carotenoids, phenolics, anthocyanins, and glucosinolates than green leaf kale (Jeon et al., 2018; Waterland et al., 2019).

The content and composition of glucosinolates are regulated by approximately 105 genes in the *Brassica oleracea* L. genome; however, these genes are differently expressed or unexpressed in different species or cultivars of *B. oleracea* (Yi et al., 2015). The functions of the key genes involved in the glucosinolate biosynthetic pathway, such as BACT, MAM, CYP79F, CYP83, UTG74B, and SOT genes, have been confirmed in *Arabidopsis*, cabbage, and broccoli (Naur et al., 2003; Piotrowski et al., 2004). Methionine elongation

is initiated by methylthioalkylmalate synthase (MAM), bile acid transporter5 (BAT5), branched chain aminotransferase (BCAT), isopropyl malate isomerase (IPMI), and isopropyl malate dehydrogenase (IPM-DH) (Sawada et al., 2009a; Sawada et al., 2009b). The core structures of glucosinolates are formed via catalysis by the CYP79 enzymes of the cytochrome P450 family (CYP79F1 and CYP79F2 for the aliphatic group, CYP79B2 and CYP79B3 for the indolic group, and CYP79A1 for aromatic glucosinolates) (Hull et al., 2000; Mikkelsen et al., 2000), CYP83 enzyme family (CYP83A1 for aliphatic and CYP83B1 for indolic) (Bak and Feyereisen, 2001), C-S lyase (SUR1) (Mikkelsen et al., 2004), glucosyltransferase (UGT74; UGT74B1 and UGT74C1 for aliphatic and UGT74B1 for indolic) (Grubb et al., 2004; Grubb et al., 2014), and sulfonyltransferases (SOTs) (SOT17 and SOT18 for aliphatic, SOT16 for indolic) (Piotrowski et al., 2004). In the present study, most of these genes were identified to be involved in glucosinolate biosynthesis in the three kale cultivars based on the KEGG pathway analysis. In addition, the glucosinolate content in the three kale cultivars showed a pattern similar to that of the expression of glucosinolate synthesis genes. For example, CYP83B1 and SOT6 are involved in the synthesis of both indolic and aromatic glucosinolates (Bak and Feyereisen, 2001; Grubb and Abel, 2006; Sønderby et al., 2010), and these genes were significantly upregulated in 'Red-Curled' kale, which corresponded to the higher content of glucobrassicin and gluconasturtiin in 'Red-Curled' kale. All identified genes in Red-Curved kale were upregulated compared to those in the green kale cultivars, which corresponded to the higher total glucosinolate content in the red cultivar than that found in the green cultivars.

In conclusion, this study revealed differences in the transcriptome and metabolome of the three kale cultivars. Eight genes involved in glucosinolate synthesis exhibited differential expression in the three cultivars, and some genes were found to be involved in secondary metabolite synthesis. Seven glucosinolates, including glucoiberin, sinigrin, gluconapin, gluconasturtiin, progoitrin, glucoraphanin, and glucobrassicin, were detected among these cultivars, and glucobrassicin was identified as the most common glucosinolate. For cultivation in vertical farms, the green 'Man-Choo' cultivar had higher growth and development and relatively higher total glucosinolate content, while the 'Red-Curled' cultivar had the lowest growth but contained the highest total glucosinolate content. Overall, metabolomic and transcriptomic analyses may provide useful information for selecting cultivars for cultivation in vertical farms.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (No. 2018R1C1B5086600).

Literature Cited

Awasthi S., and N.T. Saraswathi 2016, Elucidating the molecular interaction of sinigrin, a potent anticancer glucosinolate from cruciferous vegetables with bovine serum albumin: effect of methylglyoxal modification. J Biomol Struct Dyn 34:2224-2232. doi:10.1080/07391102.2015.1110835

Ayaz F.A., R.H. Glew, M. Millson, H.S. Huang , L.T. Chuang, C. Sanz , and S. Hayırlıoglu-Ayaz 2006, Nutrient contents of kale (*Brassica oleraceae* L. var. *acephala* DC.). Food Chem 96:572-579. doi:10.1016/j.foodchem.2005.03.011

Bak S., and R. Feyereisen 2001, The involvement of two P450 enzymes, CYP83B1 and CYP83A1, in auxin homeostasis and glucosinolate biosynthesis. Plant Physiol 127:108-118. doi:10.1104/pp.127.1.108

Burger J., and G.E. Edwards 1996, Photosynthetic efficiency, and photodamage by UV and visible radiation, in red versus green leaf coleus varieties. Plant Cell Physiol 37:395-399. doi:10.1093/oxfordjournals.pcp.a028959

Grubb C.D., and S. Abel 2006, Glucosinolate metabolism and its control. Trends Plant Sci 11:89-100. doi:10.1016/j.tplants.2005.12.006

Grubb C.D., B.J. Zipp, J. Kopycki, M. Schubert, M. Quint, E. K. Lim, D.J. Bowles, M.S.C. Pedras, and S. Abel 2014, Comparative analysis of Arabidopsis UGT 74 glucosyltransferases reveals a special role of UGT 74C1 in glucosinolate biosynthesis. Plant J 79:92-105. doi:10.1111/tpj.12541

Grubb C.D., B.J. Zipp, J. Ludwig-Müller, M.N. Masuno, T.F. Molinski, and S. Abel 2004, Arabidopsis glucosyltransferase UGT74B1 functions in glucosinolate biosynthesis and auxin homeostasis. Plant J 40:893-908. doi:10.1111/j.1365-313X.2004.02261.x

Herr I., and M.W. Büchler 2010, Dietary constituents of broccoli and other cruciferous vegetables: implications for prevention and therapy of cancer. Cancer Treat Rev 36:377-383. doi:10.1016/j.ctrv.2010.01.002

Hull A.K., R. Vij, and J.L. Celenza 2000, *Arabidopsis* cytochrome

P450s that catalyze the first step of tryptophan-dependent indole-3-acetic acid biosynthesis. Proc Natl Acad Sci 97:2379-2384. doi:10.1073/pnas.040569997

Jahangir M., H.K. Kim, Y.H. Choi, and R. Verpoorte 2009, Health-affecting compounds in *Brassicaceae*. Compr Rev Food Sci Food Saf 8:31-43. doi:10.1111/j.1541-4337.2008.00065.x

Jeon J., J.K. Kim, H. Kim H,Y.J. Kim, Y.J. Park, S.J. Kim, C.S. Kim, and S.U. Park 2018, Transcriptome analysis and metabolic profiling of green and red kale (*Brassica oleracea* var. *acephala*) seedlings. Food Chem 241:7-13. doi:10.1016/j.foodchem.2017.08.067

Kim K.H., and S.O. Chung 2018, Comparison of plant growth and glucosinolates of Chinese cabbage and kale crops under three cultivation conditions. J Biosyst Eng 43:30-36. doi:10.5307/JBE.2018.43.1.030

Kozai T. 2013, Sustainable plant factory: Closed plant production systems with artificial light for high resource use efficiencies and quality produce. Acta Hortic 1004:27-40. doi:10.17660/ActaHortic.2013.1004.2

Lännenpää M. 2014, Heterologous expression of AtMYB12 in kale (*Brassica oleracea* var. *acephala*) leads to high flavonol accumulation. Plant Cell Rep 33:1377-1388. doi:10.1007/s00299-014-1623-6

Lee G.J, J.W. Heo, C.R. Jung, H.H. Kim, J.S. Jo, J.G. Lee, G.J. Lee, S.Y. Nam, and E.Y. Hong 2016, Effects of artificial light sources on growth and glucosinolate contents of hydroponically grown kale in plant factory. Protected Hort Plant Fac 25:77-82. (in Korean) doi:10.12791/KSBEC.2016.25.2.77

Lee H.H., S.C. Yang, M.K. Lee, D.K. Ryu, S. Park, S.O. Chung, S.U. Park, and S.J. Kim 2015, Effect of developmental stages on glucosinolate contents in kale (*Brassica oleracea* var. *acephala*). Hortic Sci Technol 33:177-185. (in Korean) doi:10.7235/hort.2015.14017

Liu Z., A.H. Hirani, P.B.E. McVetty, F. Daayf, C.F. Quiros, and G. Li 2012, Reducing progoitrin and enriching glucoraphanin in *Braasica napus* seeds through silencing of the *GSL-ALK* gene family. Plant Mol Biol 79:179-189. doi:10.1007/s11103-012-9905-2

Lu N., E.L. Bernardo, C. Tippayadarapanich, M. Takagaki, N. Kagawa, and W. Yamori 2017, Growth and accumulation of secondary metabolites in perilla as affected by photosynthetic photon flux density and electrical conductivity of the nutrient solution. Front Plant Sci 8:708. doi:10.3389/fpls.2017.00708

Mayne S.T. 1996, Beta-carotene, carotenoids, and disease prevention in humans. FASEB J 10:690-701. doi:10.1096/fasebj.10.7.8635686

Mikkelsen M.D., C.H. Hansen, U. Wittstock, B.A. Halkier 2000, Cytochrome P450 CYP79B2 from Arabidopsis catalyzes the conversion of tryptophan to indole-3-acetaldoxime, a precursor of indole glucosinolates and indole-3-acetic acid. J Biol Chem 275:33712-33717. doi:10.1074/jbc.M001667200

Mikkelsen M.D., P. Naur, and B.A. Halkier 2004, *Arabidopsis* mutants in the C–S lyase of glucosinolate biosynthesis establish a critical role for indole-3-acetaldoxime in auxin homeostasis. Plant J 37:770-777. doi:10.1111/j.1365-313X.2004.02002.x

Naur P., B.L. Petersen, M.D. Mikkelsen, S. Bak, H. Rasmussen, C.E. Olsen, and B.A. Halkier 2003, CYP83A1 and CYP83B1, two nonredundant cytochrome P450 enzymes metabolizing oximes in the biosynthesis of glucosinolates in *Arabidopsis*. Plant Physiol 133:63-72. doi:10.1104/pp.102.019240

Neugart S., S. Baldermann, F.S. Hanschen, R. Klopsch, M. Wiesner-Reinhold, and M. Schreiner 2018, The intrinsic quality of brassicaceous vegetables: How secondary plant metabolites are affected by genetic, environmental, and agronomic factors. Sci Hortic 233:460-478. doi:10.1016/j.scienta.2017.12.038

Nguyen T.K.L., and M.M. Oh 2021, Physiological and biochemical responses of green and red perilla to LED-based light. J Sci Food Agric 101:240-252. doi:10.1002/jsfa.10636

Palani K., B. Harbaum-Piayda, D. Meske, J.K. Keppler, W. Bockelmann, K.J. Heller, and K. Schwarz 2016, Influence of fermentation on glucosinolates and glucobrassicin degradation products in sauerkraut. Food Chem 190:755-762. doi:10.1016/j.foodchem.2015.06.012

Piotrowski M., A. Schemenewitz, A. Lopukhina, A. Müller, T. Janowitz, E.W. Weiler, and C. Oecking 2004, Desulfoglucosinolate sulfotransferases from *Arabidopsis thaliana* catalyze the final step in the biosynthesis of the glucosinolate core structure. J Biol Chem 279:50717-50725. doi:10.1074/jbc.M407681200

Sawada Y., A. Kuwahara, M. Nagano, T. Narisawa, A. Sakata, K. Saito, and M. Y. Hirai 2009a, Omics-based approaches to methionine side chain elongation in Arabidopsis: characterization of the genes encoding methylthioalkylmalate isomerase and methylthioalkylmalate dehydrogenase. Plant Cell Physiol 50:1181-1190. doi:10.1093/pcp/pcp079

Sawada Y., K. Toyooka, A. Kuwahara, A. Sakata, M. Nagano, K. Saito, and M.Y. Hirai 2009b, Arabidopsis bile acid:sodium symporter family protein 5 is involved in methionine-derived glucosinolate biosynthesis. Plant Cell Physiol 50:1579-1586. doi:10.1093/pcp/pcp110

Smillie R.M., and S.E. Hetherington 1999, Photoabatement by anthocyanin shields photosynthetic systems from light stress. Photosynthetica 36:451-463. doi:10.1023/A:1007084321859

Sønderby I.E., F. Geu-Flores, and B.A. Halkier 2010, Biosynthesis of glucosinolates–gene discovery and beyond. Trends Plant Sci 15:283-290. doi:10.1016/j.tplants.2010.02.005

Waterland N.L., Y. Moon, J.C. Tou, D.A. Kopsell, M.J. Kim, and S. Park 2019, Differences in leaf color and stage of development at harvest influenced phytochemical content in three cultivars of kale (*Brassica oleracea* L. and *B. napus*). J

Agric Sci 11:14-21. doi:10.5539/jas.v11n3p14
Yan X., and S. Chen 2007, Regulation of plant glucosinolate metabolism. Planta 226:1343-1352. doi:10.1007/s00425-007-0627-7.
Yi G.E., A.H.K. Robin, K. Yang, J.I. Park, J.G. Kang, T.J. Yang, and I.S. Nou 2015, Identification and expression analysis of glucosinolate biosynthetic genes and estimation of glucosinolate contents in edible organs of *Brassica oleracea* subspecies. Molecules 20:13089-13111. doi:10.3390/molecules200713089

수직농장에서 자란 케일(*Brassica oleracea* var. *acephala*) 품종에 따른 글루코시놀레이트 함량의 변화 및 전사체 분석

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적 요. 케일(*Brassica oleracea* var. *acephala*)은 필수 아미노산, 비타민, 미네랄과 같은 수많은 영양소를 함유하고 특히 글루코시놀레이트가 풍부하기 때문에 전 세계적으로 가장 많이 소비되는 잎 채소 중 하나이다. 그러나 케일 품종 간의 글루코시놀레이트 합성과 관련된 유전자 발현에 대한 연구는 미비한 실정이다. 본 연구에서는 전사체 및 대사체 분석을 사용하여 식물공장에서 재배된 녹색(만추 및 맛짱) 및 적색 케일 품종(적곱슬)을 포함한 3 가지 케일 품종에서 글루코시놀레이트를 조사하였다. 재배 후 6주된 녹색 케일 품종의 생육 및 발달이 적색 케일 품종에 비해 높았다. High-performance liquid chromatography (HPLC) 분석에서 7가지 글루코시놀레이트를 분석하였다; 만추 품종에서는 5종의 글루코시놀레이트가, 맛짱과 적곱슬 품종에서는 4종의 글루코시놀레이트가 분류되었다. Glucobrassicin은 3가지 케일 품종에서 가장 높은 글루코시놀레이트 였다. 총 글루코시놀레이트 함량은 적곱슬 품종에서 가장 높았다. 전사체 분석에서는 8개의 유전자가 글루코시놀레이트 합성에 관여됨을 확인할 수 있었다. 이러한 결과는 케일 품종에 따라 글루코시놀레이트 함량과 축적 패턴이 다르다는 것을 시사한다.

추가 주제어 : 글루코시놀레이트, 생육, 케일, 대사체, 식물공장, 전사체