Article

Effects of Short-Term Root Cooling before Harvest on Yield and Food Quality of Chinese Broccoli (*Brassica oleracea* var. *Alboglabra* Bailey)

Fang He [1,*]**, Björn Thiele** [1,2]**, David Kraus** [1]**, Souhaila Bouteyine** [1]**, Michelle Watt** [1,3,4]**, Thorsten Kraska** [3]**,
Ulrich Schurr** [1] **and Arnd Jürgen Kuhn** [1]

[1] Forschungszentrum Jülich, IBG-2: Plant Science, Wilhelm-Johnen-Straße, 52428 Jülich, Germany;
b.thiele@fz-juelich.de (B.T.); d.kraus@fz-juelich.de (D.K.); Souha.bouteyine@outlook.fr (S.B.);
watt.m@unimelb.edu.au (M.W.); u.schurr@fz-juelich.de (U.S.); a.kuhn@fz-juelich.de (A.J.K.)
[2] Forschungszentrum Jülich, IBG-3: Agrosphere, Wilhelm-Johnen-Straße, 52428 Jülich, Germany
[3] Institute of Crop Science and Resource Conservation, Faculty of Agriculture, Campus Klein-Altendorf 1,
University of Bonn, 53359 Rheinbach, Germany; kraska@uni-bonn.de
[4] School of BioSciences, University of Melbourne, Parkville, Melbourne 3010, Australia
[*] Correspondence: f.he@fz-juelich.de

Abstract: Vegetable product quality is an important consideration for consumers. Long-term root cooling could improve certain food quality of horticultural crops, but often comes at the expense of reduced shoot biomass or yield. Since few studies have investigated how fast Chinese broccoli (*Brassica oleracea* var. *alboglabra* Bailey) responds to changes of root temperature, we shortened the duration of the root cooling treatment to one week before harvest to make the production system more effective. The aim of this study was to improve the food quality of Chinese broccoli without causing deleterious effects on plant growth and yield. The seedlings were cultivated hydroponically at two root temperatures (10 and 20 °C) during the last week prior to harvest in summer 2018 (Exp-1) and autumn 2019 (Exp-2). Plant growth, yield, physiological variables, soluble sugars, total chlorophyll, glucosinolates and mineral elements concentration were examined. The results showed that the yield reduction was alleviated compared to results over the long-term. Specifically, yield was not affected by root cooling in Exp-1 and reduced by 18.9% in Exp-2 compared to 20 °C. Glucose and fructose concentrations of the leaves were increased when the root temperature was 10 °C in both experiments with a more pronounced impact in Exp-2. In addition, root cooling produced a significant accumulation of individual glucosinolates, such as progoitrin, gluconapin, 4-methoxyglucobrassicin and 4-hydroxyglucobrassicin, in the stems of Exp-1 and the leaves of Exp-2. Minerals, such as N, showed reductions in the shoot, but accumulation in the root. Therefore, compared to long-term root cooling, short-term (one week) reduction of the root temperature is more economical and could help improve certain quality characteristics of Chinese broccoli with less or even no yield reduction.

Keywords: yield; biomass; glucosinolates; soluble sugars; chlorophyll; starch; minerals

1. Introduction

Chinese broccoli (*Brassica oleracea* var. *alboglabra* Bailey), also known as Chinese kale and Gai lan, is original from South Asia and one of the most popular leaf vegetables in these regions [1]. Chinese broccoli is grown for its bolting stems and tender rosette leaves as the main edible parts [2]. Due to its flavor and high concentration of health-promoting phytochemicals and minerals, such as glucosinolates, chlorophyll, and essential mineral elements, Chinese broccoli has gained wide recognition as a healthy vegetable [2]. Notably, glucosinolates are the characteristic natural antioxidants in Brassica vegetables and a group of sulfur-and nitrogen-containing compounds derived from different amino acids [3]. Glucosinolates alone have limited health benefits for humans, but the hydrolysis product

isothiocyanates are proven to exhibit cholesterol-lowering, anti-carcinogenic and anti-mutagenic activity, and therefore consumption of food rich in glucosinolates is associated with reduced risk of cancer and other chronic diseases [4,5]. Sugar levels, together with glucosinolates, determine the flavor and acceptance of Chinese broccoli by the consumer [6] In addition, Chinese broccoli, like other leaf vegetables, is believed to provide a modest source of essential mineral elements including K, Ca and Mg, for well-balanced diets [7].

Root temperature plays a critical role in plant growth and influences the concentration of some primary and most secondary metabolites [8–11]. Reactive oxygen species (ROS) are produced under temperature stress and as an antioxidant defense mechanism, the biosynthesis of bioactive compounds in plants is provoked to counteract the oxidative damage caused by ROS [12]. Applying temperature stresses during cultivation is, therefore, an effective method to improve the bioactive compound levels of horticultural plants. Furthermore, root temperature can easily be controlled in greenhouse cultivation. Several studies have shown that long-term root cooling improves nutritional quality by increasing the levels of beneficial functional plant constituents. For example, after a 24-day root temperature treatment, the rosmarinic acid and acacetin concentration of *Agastache rugosa* was highest at 10 °C root temperature [13]. In hydroponically grown carrots, a 14-day treatment with an elevated nutrient solution temperature increased the total phenolic compounds and soluble solid content [10]. Similarly, our previous studies of the effects of root temperature on food quality of Chinese broccoli [14] and cocktail tomato [15] have shown that long-term root cooling can be used as a strategy to accumulate high levels of phytochemicals such as sugar, chlorophyll, lycopene and glucosinolates with potential practical applications.

However, cold temperature is one of the most devastating abiotic stress causing agricultural loss in horticultural crops [16,17]. For example, long-term root cooling caused 8–21% yield loss in cocktail tomato [15] and 21% loss in Chinese broccoli [14]. Under long-term cold stress conditions, photosynthesis, root respiration, water uptake, and hormone signaling were altered [18–20]. Additionally, the reallocation of resources to accumulate phytochemicals was at the expense of growth [21], resulting in a reduced vegetable yield—an undesirable outcome for growers. Ogawa et al. [22] showed that a short-term treatment (6 days) of red perilla (*Perilla frutescens*) with a low temperature (10 °C) nutrient solution increased perillaldehyde and rosmaricnic acid content, but dry weight of leaves was unaffected. In addition, a one-week 5 °C root temperature treatment improved the concentration of ascorbic acid and soluble sugar of spinach, while the fresh weight reduction of the shoot was lower than that obtained from a constant two-week 5 °C treatment [23]. We hypothesized that short-term root cooling could trigger physiological stress responses of the plant resulting in higher contents of desired beneficial compounds without interfering heavily with crop performance. Moreover, short-term root cooling involves less energy and is therefore more economical. Our previous findings revealed that the effects of root cooling vary between growing seasons and years [14]. As Chinese broccoli is grown all year round and under diverse climatic conditions, products may have different phytochemical concentrations as a result [24]. To account for the climatic factors, we conducted two separate experiments in summer (Exp-1) and autumn (Exp-2). The objective of the present study was to investigate whether short-term (one-week) root cooling immediately before harvest promotes the accumulation of glucosinolates, sugar, and total chlorophyll without affecting yield.

2. Materials and Methods

2.1. Plant Materials and Experimental Setup

Experiments were conducted under greenhouse conditions (Forschungszentrum Jülich, Germany), in August 2018 (Exp-1) and October 2019 (Exp-2). Chinese broccoli seeds were provided by Guangdong Academy of Agricultural Sciences (Guangdong, China). Before sowing, seeds were sanitized in boiling distilled water for 5–10 min and germinated in paper soaked with distilled water in darkness at room temperature (25/20 °C day/night).

Six days after sowing (DAS), the seedlings were transplanted into six cm diameter pots filled with sand irrigated with 25% Hoagland solution (1.25 mM $CaNO_3$, 1.25 mM KNO_3, 0.5 mM $MgSO_4$, 0.25 mM KH_2PO_4, 22.4 μM Fe(EDTA), 2.5 μM $MnCl_2$, 0.25 μM $CuSO_4$, 0.25 μM $ZnSO_4$, 12.5 μM H_3BO_3, 0.125 μM Na_2MoO_4) [25]. On the third true leaf stage (31 DAS), 32 uniform seedlings were transplanted into 8 containers ($28 \times 43 \times 17$ cm) filled with 50% Hoagland solution (2.5 mM $CaNO_3$, 2.5 mM KNO_3, 1 mM $MgSO_4$, 0.5 mM KH_2PO_4, 44.8 μM Fe(EDTA), 5 μM $MnCl_2$, 0.5 μM $CuSO_4$, 0.5 μM $ZnSO_4$, 25 μM H_3BO_3, 0.25 μM Na_2MoO_4) in the hydroponic system. Each container held four plants. Constant pH (6.0–6.5) and EC (1.1–1.2 ms/cm) were guaranteed. New nutrient solution was added every day, and all of the nutrient solution was changed weekly. Plants were placed in the greenhouse maintained at relative humidity around 50% and daily 16 h light and receiving natural light. Light and air temperature during the growth were recorded by the climate station within the greenhouse.

Root temperature treatment was started one week before harvest (45 DAS) by circulating the nutrition solution through a thermostat (Oceanrunner OR1200, Aqua Medic, Bissendorf, Germany). Root temperature was set at 20 °C (control) vs. 10 °C (cool).

All the plants were harvested at the same time (51 DAS) at the commercial harvest stage (the height of the stem is the same as that of the leaves) [26]. Each plant was considered a replicate. After harvest, shoot fresh weight (FW), shoot height, root FW and root length were recorded. The upper plant part (including the main stem with terminal floral buds and 6–7 leaves) above the fifth node was regarded as consumable and the weight was recorded as yield. Bolting stem diameter was measured at the thickest part of the marketable portion. The leaves and stems were cut and divided into two parts. One part was immediately flash-frozen in liquid nitrogen and stored at −80 °C until further phytochemical analysis. The other part was dried at 65 °C until constant weight for dry weight (DW) and element analysis. Root DW was recorded directly after drying. Shoot DW was calculated as the sum of DW of leaves and stems. Shoot ratio based on dry weight was calculated based on the following formula,

$$\text{Shoot ratio} = (\text{Shoot DW})/(\text{Shoot DW} + \text{Root DW}) \times 100\%$$

2.2. Evaluation of Photosynthesis, Transpiration, Stomatal Conductance and Leaf Temperature

In Exp-2, net photosynthesis, transpiration, stomatal conductance and leaf temperature were determined by a portable photosynthesis system, LI-6400 (LiCor, Lincoln, NE, USA). The fully developed youngest mature leaf of each plant was measured at the photosynthetically active radiation (PAR) of 400 μmol m^{-2} s^{-1}, a CO_2 concentration of 400 μmol mol^{-1}, cuvette air temperature of 21 °C and relative humidity between 50 and 60%. Measurements were taken from 10:00 to 12:00 h at 47 and 50 DAS, representing 3 d and 6 d after treatment initiated, respectively. Due to the schedule and maintenance of the device, measurements of these parameters in Exp-1 were missed.

2.3. Soluble Sugar, Total Chlorophyll and Starch Analysis

In both experiments, extraction of soluble sugar (glucose, fructose and sucrose), total chlorophyll and starch from frozen Chinese broccoli leaf and stem samples was performed based on the method described by Viola and Davies [27] with slight modifications described by He et al. [15]. Briefly, 400 μL 80% (*v/v*) ethanol was added to 50 mg of homogenized frozen powder. Samples were incubated at 80 °C for 15 min, centrifuged at 13,200 rpm for 3 min and the supernatant was collected. The same procedure was repeated with 400 μL 50% (*v/v*) ethanol, 200 μL 80% (*v/v*) ethanol and 200 μL 80% (*v/v*) ethanol consecutively until pellets were colorless. All supernatants were pooled, homogenized and directly measured, or stored at −80 °C until further analysis.

Total chlorophyll concentration was immediately determined after extraction with a microplate reader (Synergy™ 2 Multi-Mode, BioTek, Winooski, VT, USA) at 652 nm. Glucose, fructose and sucrose concentrations were determined at 340 nm based on Viola and Davies [27] with slight modification described by He et al. [15]. Starch analysis was

conducted on the pellet after sugar extraction. The pellets were washed with distilled water first and then autoclaved at 120 °C for 90 min. After incubating in sodium acetate, α-amylase and amyloglucosidase solution (Roche Diagnostics, Basel, Switzerland) at 37 °C, starch was hydrolyzed to glucose. The determination of starch concentration was the same enzymatic reaction as glucose. Each sample was extracted and analyzed in duplicate and the results were reported as mg/g FW.

2.4. Mineral Elements Quantification

Dried Chinese broccoli leaf, stem samples of Exp-1 and Exp-2 and root samples of Exp-2 were finely ground in a pebble mill (MM400, Retsch, Haan, Germany). Root samples of Exp-1 were not analyzed due to mishandling during the preparation. Carbon and nitrogen were analyzed with a CHNS-Analyzer (Leco CHNS-932, St. Joseph, MO, USA). Mg, K, Ca and P were analyzed by integration of the dried sample with HNO_3, H_2O_2 and HF (Hydrogen Fluoride) first in a microwave and then by ICP-OES (inductively coupled plasma with optical emission spectroscopy, Agilent 7500ce, Waldbronn Germany) after dilution. The concentration was expressed as % of DW. Moreover, shoot/root C, N, Ca, K, Mg, P content ratio of plants were calculated as the content of each mineral element in the shoot divided by the same element content in root.

2.5. Glucosinolates Analysis

Intact individual glucosinolates were determined as described by He et al. [14]. Briefly, approximately 60 mg of homogenized frozen leaf and stem samples of Exp-1 and Exp-2 were used for the extractions. The powder was mixed with 1.5 mL of 80% (v/v) methanol at room temperature for 30 min, vortexed for another 30 min and centrifuged for 10 min. After adding another 1.5 mL of 80% (v/v) methanol, the same procedure was repeated. The supernatants were pooled and evaporated in a vacuum concentrator (Eppendorf Concentrator 5301, Hamburg, Germany) at a temperature below 30 °C. The dried extract was redissolved in 240 μL of 50% (v/v) methanol and filtered through 0.2 μm filter (Whatman, PTFE (Polytetrafluoroethylene), 4 mm, Dassel, Germany) before injecting the LC-MS systems (1260, Agilent Technologies). Compounds were separated on a 150 × 3 mm, 3 μm particle size, Nucleodur C18 Gravity-SB column (Macherey-Nagel, Düren, Germany). Mobile phase consisted of water containing 0.1% formic acid (phase A) and acetonitrile containing 0.1% formic acid (phase B). The gradient solvent system was at a constant flow rate of 1 mL/min as follows: 100% A, linear gradient to 52.3% A over 22 min, isocratic at 100% B for 3 min and finally equilibration at the initial condition (100% A) for 5 min. Authentic standard individual glucosinolates (Phytoplan Diehm and Neuberger GmbH, Heidelberg, Germany) were dissolved in 50% (v/v) methanol for calibration. Individual glucosinolates were identified on the basis of retention time and m/z ratio compared with standards. The concentration of total glucosinolates, total aliphatic glucosinolates, total indolic glucosinolates were calculated by adding up individual glucosinolates of the respective category and expressed as μmol per 100 mg of Chinese broccoli FW.

2.6. Statistical Analysis

Each plant was regarded as one replicate. For each treatment, there were 16 replicates. Due to the different environmental factors in each experiment, data from each trial were analyzed separately. All results were expressed as the mean $\pm$ standard deviation. Statistical significance between the control and cool group was analyzed by Student's t-test. Differences at $p \leq 0.05$ were considered statistically significant and indicated in bold numbers.

3. Results

3.1. Greenhouse Climate Conditions

The experiments were conducted in summer 2018 and autumn 2019 in the greenhouse, and the climate conditions were affected by outdoor light and air temperature (Figure 1).

Fluctuations of air temperature and light were consistent for each experiment, but average daily air temperature and light during Exp-2 were lower than those of Exp-1.
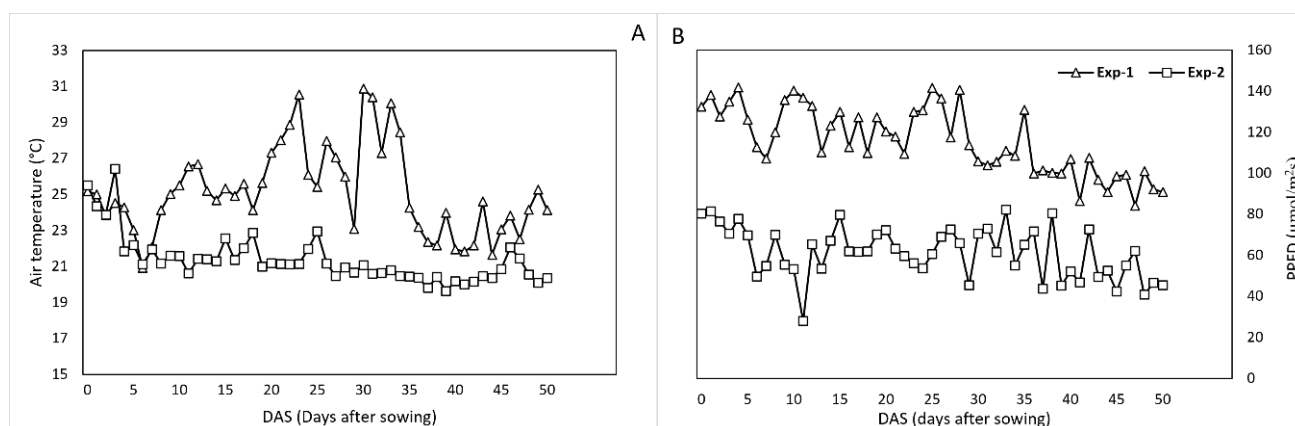

Figure 1. Daily average air temperature (**A**) and PPFD (photosynthetic photon flux density) (**B**) recorded during Exp-1 and Exp-2 to test the influence of rooting temperature on Chinese broccoli growth and quality in a glasshouse. DAS: days after sowing.

3.2. Biomass and Yield

In both experiments, only the fresh weight of shoots was significantly affected by the root temperature (Table 1). The production of dry matter was not affected in the shoot ($p > 0.05$). The shoot (including stems and leaves) above the fifth node was considered consumable and the weight of it was recorded as the yield. The yield in Exp-2 was reduced by 18.9% ($p = 0.009$), while no differences were observed in Exp-1. Shoot height, bolting stem diameter and shoot ratio (% based on DW) were not statistically reduced by root temperature. Further, root cooling had no influence ($p > 0.05$) on the growth parameters of root systems (FW, DW and length).

Table 1. Yield, shoot and root fresh weight (FW), shoot and root dry weight (DW), shoot height, bolting stem diameter, root length and shoot ratio of Chinese broccoli under different root temperatures in Exp-1 and Exp-2. Significant differences ($p < 0.05$) were indicated in bold.

Experiment		Exp-1			Exp-2		
	Treatment	10 °C	20 °C	*p*-Value	10 °C	20 °C	*p*-Value
Shoot	Yield (g)	109.72 ± 13.74	120.97 ± 13.90	0.062	89.31 ± 17.91	110.25 ± 16.61	**0.009**
	FW (g)	150.33 ± 18.67	166.91 ± 18.16	**0.038**	117.17 ± 20.93	140.13 ± 16.76	**0.009**
	DW (g)	11.5 ± 1.22	12.49 ± 1.48	0.052	10.39 ± 1.79	11.34 ± 1.40	0.177
	Height (cm)	30.9 ± 2.64	33.1 ± 3.40	0.091	30.55 ± 4.84	32.58 ± 3.45	0.264
	Bolting stem diameter (mm)	17.24 ± 0.89	17.86 ± 0.80	0.087	14.68 ± 1.31	15.47 ± 1.14	0.139
	Shoot ratio %	93.02 ± 0.61	93.19 ± 0.93	0.617	97.01 ± 0.41	96.90 ± 0.34	0.502
Root	FW (g)	14.47 ± 2.70	16.56 ± 2.33	0.058	5.50 ± 1.15	6.52 ± 1.59	0.090
	DW (g)	0.85 ± 0.13	0.91 ± 0.13	0.275	0.32 ± 0.06	0.36 ± 0.07	0.090
	Length (cm)	30.35 ± 4.45	31.31 ± 3.93	0.589	27.95 ± 5.81	33.17 ± 6.58	0.056

3.3. Net Photosynthesis, Transpiration, Stomatal Conductance and Leaf Temperature

In Exp-2, net photosynthesis, transpiration and stomatal conductance were recorded three days (47 DAS) and six days (50 DAS) after the treatment started (Table 2) to understand the gas exchange. Leaf transpiration rate and stomatal conductance were higher at low root temperature, but leaf temperature and net photosynthesis rate were not affected three days after the treatment (47 DAS). After another 3 days of root cooling (50 DAS), no differences were detected in the four parameters.

Table 2. Effect of root temperature treatment on leaf temperature, transpiration rate, stomatal conductance and net photosynthetic rate of Chinese broccoli at two dates (47 DAS and 50 DAS) after treatment started in Exp-2. Significant differences ($p < 0.05$) were indicated in bold.

	47 DAS			50 DAS		
	10 °C	20 °C	*p*-Value	10 °C	20 °C	*p*-Value
Leaf temperature (°C)	22.29 ± 0.53	22.42 ± 0.43	0.588	22.17 ± 0.36	22.05 ± 0.46	0.568
Transpiration rate (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$)	7.01 ± 0.71	5.69 ± 1.12	**0.016**	5.60 ± 0.66	6.30 ± 0.65	0.051
Stomatal conductance (mol $H_2O \cdot m^{-2} \cdot s^{-1}$)	0.63 ± 0.10	0.48 ± 0.16	**0.043**	0.46 ± 0.08	0.54 ± 0.11	0.142
Net photosynthesis rate (µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)	8.84 ± 0.45	8.66 ± 0.57	0.486	9.03 ± 1.49	9.35 ± 0.66	0.587

3.4. Soluble Sugars, Starch and Total Chlorophyll

Calculations made for these factors were based on fresh weight because fresh products are generally consumed. The response of soluble sugars to root temperature treatment varied by season and tissue (Table 3). In general, leaves of Chinese broccoli had a higher concentration of soluble sugars under root cooling treatment. The concentration of glucose and fructose in leaves increased by 20.5% and 21.7%, respectively, when the roots were exposed to 10 °C in Exp-1. In Exp-2, the glucose and fructose levels in leaves almost doubled. In both experiments, the sucrose level of leaves was not significantly affected by root temperature. In Exp-1, no significant changes in stem sugar levels were observed in root cooling-treated plants, while in Exp-2, the three soluble sugar concentrations increased by 33.4–87.5% and the total soluble sugar concentration increased by 39.8%. Compared to soluble sugars, the accumulation of starch was only detected in the leaves of Exp-2, with a 68.8% increase. In Exp-1, the concentration of total chlorophyll in leaves increased by around 5.8% at a root temperature of 10 °C compared to 20 °C (Table 3).

Table 3. Soluble sugars, total chlorophyll, and starch concentration (mg/g FW) of leaves and stems of Chinese broccoli under different root temperatures and harvest dates in Exp-1 and Exp-2. Significant differences ($p < 0.05$) were indicated in bold. FW: fresh weight.

(mg/g FW)	Treatment	Exp-1				Exp-2			
		10 °C	20 °C	Change [%]	*p*-Value	10 °C	20 °C	Change [%]	*p*-Value
Leaf	Glucose	3.12 ± 0.68	2.59 ± 0.40	**+20.5**	**0.039**	0.92 ± 0.27	0.43 ± 0.22	**+114.0**	**<0.001**
	Fructose	3.37 ± 0.59	2.77 ± 0.31	**+21.7**	**0.009**	1.15 ± 0.31	0.52 ± 0.23	**+121.2**	**<0.001**
	Sucrose	1.11 ± 0.38	0.86 ± 0.21		0.072	0.54 ± 0.31	0.52 ± 0.33		0.907
	Chlorophyll	6.02 ± 0.38	5.69 ± 0.28	**+5.8**	**0.032**	5.42 ± 0.54	5.31 ± 0.32		0.559
	Starch	1.52 ± 0.65	1.84 ± 1.10		0.422	0.54 ± 0.23	0.32 ± 0.17	**+68.8**	**0.018**
	Soluble sugar	6.96 ± 2.54	5.69 ± 1.92		0.183	2.61 ± 0.50	1.47 ± 0.64	**+77.6**	**<0.001**
Stem	Glucose	7.35 ± 0.75	7.51 ± 0.71		0.612	9.50 ± 0.89	7.12 ± 1.38	**+33.4**	**<0.001**
	Fructose	5.62 ± 0.59	5.75 ± 0.64		0.608	7.96 ± 0.69	5.95 ± 1.03	**+33.8**	**<0.001**
	Sucrose	2.40 ± 0.40	2.66 ± 0.50		0.180	3.15 ± 0.56	1.68 ± 0.46	**+87.5**	**<0.001**
	Chlorophyll	0.37 ± 0.05	0.36 ± 0.04		0.668	0.34 ± 0.05	0.31 ± 0.11		0.498
	Starch	0.21 ± 0.04	0.23 ± 0.07		0.422	0.19 ± 0.05	0.15 ± 0.06		0.076
	Soluble sugar	14.09 ± 4.70	15.92 ± 1.36		0.217	20.61 ± 1.95	14.74 ± 2.43	**+39.8**	**<0.001**

3.5. Glucosinolates

The glucosinolate patterns of the stems and leaves were similar in both Exp-1 and Exp-2 (Table 4). Two groups of glucosinolates were identified in Chinese broccoli leaves and stems, and the major glucosinolates in the shoots of Chinese broccoli were aliphatic groups, with gluconapin as the most abundant in both stems and leaves. The predominant indolic glucosinolate was glucobrassicin, with higher levels in leaves. In both experiments, lower root temperature (10 °C) had a positive influence on the concentration of glucosinolates. In Exp-1, the root cooling effects were more pronounced in stems than in leaves. Most of the glucosinolates in leaves showed no significant increase ($p > 0.05$), such as sinigrin,

glucoraphanin and gluconapin. In stems, progoitrin and gluconapin of the aliphatic group, and 4-methoxyglucobrassicin and 4-hydroxyglucobrassicin of the indolic group increased by 49.4%, 24.3%, 18.7%, and 32.5%, respectively, at 10 °C compared to 20 °C root temperature. The increased percentage of total aliphatic and indolic glucosinolate concentrations under low root temperature was almost the same at around 21%. Results of Exp-2 revealed that the concentration of most glucosinolates in leaves was higher at 10 °C than 20 °C root temperature, while in stems, only 4-methoxyglucobrassicin was increased significantly at around 22.2%. The total aliphatic glucosinolates and indolic glucosinolates concentrations of leaves were enhanced, with a similar increase at around 43% when the roots were exposed to 10 °C. No statistically significant differences were detected for other individual or total glucosinolates ($p > 0.05$) in stems.

3.6. Mineral Elements

A comparison of the mineral elements in the cooled and control group in both experiments revealed that root cooling affected the mineral concentration of Chinese broccoli (leaves, stems and roots) (Table 5). Under root cooling in Exp-1, the carbon concentration was not affected in leaves and stems ($p > 0.05$). N and Mg levels were reduced at 10 °C root temperature by around 6% and 9%, respectively, compared to 20 °C in leaves. In stems, the concentrations of N and Mg were not affected. The concentration of P was increased in leaves by approximately 8% and reduced by 14.3% in stems at lower root temperatures. The concentration of K significantly reduced in both stems ($p = 0.017$) and leaves ($p < 0.001$) at 10 °C root temperature. In Exp-2, Chinese broccoli grown at the lower root temperature accumulated 3% more carbon in leaves and stems compared to the control group. Concentrations of N, P, K, Ca and Mg were all reduced at 10 °C in stems and leaves, except for K in leaves. Contrary to the reduction in shoots, N and P concentrations in roots were increased at 10 °C by 16.1% and 28.1%, respectively.

The shoot/root content ratio of minerals in Exp-2 was significantly affected for N only (Figure 2). It could be observed that higher root temperature resulted in a higher root/shoot content ratio except for C where a higher ratio was observed at lower root temperature.
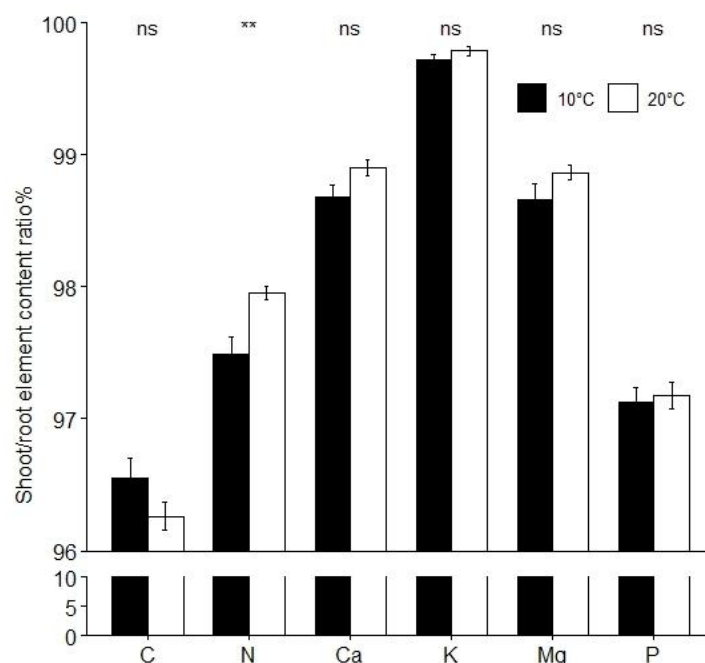

Figure 2. Shoot/root C, N, Ca, K, Mg, P content ratio of plants grown at two root temperature treatments (10 and 20 °C) in Exp-2. Mean and standard deviation were shown. Statistical analysis was performed by *t*-test. Levels of significance were represented by: ns not significant, ** $0.001 < p < 0.01$.

Table 4. Total, aliphatic, indolic and individual glucosinolates concentrations (μmol/100 g FW) in the leaves and stems of Chinese broccoli under different root temperatures and harvest dates in Exp-1 and Exp-2. Significant differences ($p < 0.05$) were indicated in bold. FW: fresh weight.

(μmol/100 g FW)		Exp-1				Exp-2			
	Treatment	10 °C	20 °C	Change [%]	*p*-Value	10 °C	20 °C	Change [%]	*p*-Value
Leaf									
	Aliphatic								
	Sinigrin	28.87 ± 7.31	24.32 ± 11.25		0.276	16.72 ± 4.81	12.49 ± 2.55	**+33.9**	**0.020**
	Progoitrin	2.47 ± 1.03	2.61 ± 1.72		0.821	1.56 ± 0.66	1.31 ± 0.34		0.280
	Glucoraphanin	7.27 ± 3.69	6.30 ± 4.37		0.582	3.40 ± 1.61	1.61 ± 0.91	**+111.2**	**0.005**
	Gluconapin	69.86 ± 21.84	58.45 ± 30.29		0.325	49.70 ± 17.22	34.39 ± 8.37	**+44.5**	**0.018**
	Glucoiberin	3.05 ± 1.38	2.60 ± 1.47		0.477	1.38 ± 0.73	0.80 ± 0.35	**+72.5**	**0.030**
	Glucoalyssin	0.15 ± 0.05	0.13 ± 0.06		0.383	0.08 ± 0.03	0.05 ± 0.02	**+60.0**	**0.026**
	Total	111.66 ± 34.66	94.42 ± 46.74		0.338	72.84 ± 23.70	50.65 ± 11.62	**+43.8**	**0.014**
	Indolic								
	Glucobrassicin	14.56 ± 6.12	12.59 ± 7.38		0.502	6.28 ± 2.52	4.31 ± 0.94	**+45.7**	**0.031**
	4-Methoxyglucobrassicin	0.81 ± 0.12	0.76 ± 0.16		0.501	1.03 ± 0.26	0.74 ± 0.13	**+39.2**	**0.006**
	Neoglucobrassicin	0.80 ± 0.52	0.79 ± 0.61		0.988	1.37 ± 0.24	1.09 ± 0.12		0.552
	4-Hydroxyglucobrassicin	2.37 ± 0.72	1.96 ± 0.65		0.179	1.87 ± 0.85	1.74 ± 1.06	**+7.5**	**0.004**
	Total	18.53 ± 7.03	16.10 ± 8.52		0.474	8.92 ± 2.96	6.35 ± 1.23	**+40.5**	**0.019**
	Total	130.19 ± 39.55	110.52 ± 54.71		0.347	81.76 ± 26.32	57.01 ± 12.37	**+43.4**	**0.013**
Stem									
	Aliphatic								
	Sinigrin	23.75 ± 5.36	20.80 ± 4.00		0.142	18.75 ± 4.86	16.31 ± 8.73		0.415
	Progoitrin	6.32 ± 1.41	4.23 ± 1.19	**+49.4**	**<0.001**	2.79 ± 1.03	3.19 ± 1.39		0.438
	Glucoraphanin	11.23 ± 2.26	9.92 ± 2.39		0.182	7.80 ± 2.50	7.33 ± 5.08		0.777
	Gluconapin	77.15 ± 18.74	62.08 ± 14.61	**+24.3**	**0.039**	51.51 ± 20.01	47.74 ± 28.87		0.718
	Glucoiberin	2.63 ± 0.53	2.21 ± 0.64		0.098	1.87 ± 0.85	1.74 ± 1.06		0.759
	Glucoalyssin	0.17 ± 0.04	0.15 ± 0.02		0.099	0.12 ± 0.04	0.10 ± 0.06		0.420
	Total	121.24 ± 25.29	99.38 ± 21.67	**+22.0**	**0.033**	82.84 ± 28.11	76.42 ± 44.80		0.683
	Indolic								
	Glucobrassicin	4.18 ± 0.88	4.03 ± 0.90		0.687	3.54 ± 1.38	3.56 ± 1.81		0.971
	4-Methoxyglucobrassicin	2.41 ± 0.33	2.03 ± 0.32	**+18.7**	**0.009**	2.37 ± 0.42	1.94 ± 0.52	**+22.2**	**0.039**
	Neoglucobrassicin	2.57 ± 0.71	2.19 ± 0.55		0.153	0.87 ± 0.46	0.96 ± 0.49		0.657
	4-Hydroxyglucobrassicin	3.30 ± 0.77	2.49 ± 0.45	**+32.5**	**0.006**	2.89 ± 0.85	2.36 ± 1.30		0.258
	Total	12.46 ± 2.19	10.74 ± 1.67	**+16.0**	**0.042**	9.67 ± 2.39	8.82 ± 3.73		0.522
	Total	133.70 ± 27.12	110.12 ± 22.85	**+21.4**	**0.031**	92.50 ± 30.34	85.24 ± 48.29		0.668

Table 5. Element concentration (% DW) of leaves, stems and roots of Chinese broccoli from different root temperatures and harvest dates in Exp-1 and Exp-2. Significant differences ($p < 0.05$) were indicated in bold. DW: dry weight.

(%)		Exp-1				Exp-2			
		10 °C	20 °C	Change [%]	*p*-Value	10	20 °C	Change [%]	*p*-Value
Leaf	C	39.43 ± 0.63	39.32 ± 0.95		0.727	34.92 ± 0.83	33.79 ± 0.76	**+3.3**	**0.003**
	N	5.84 ± 0.20	6.21 ± 0.22	**−6.0**	**<0.001**	5.92 ± 0.10	6.27 ± 0.23	**−5.6**	**<0.001**
	P	0.67 ± 0.04	0.62 ± 0.05	**+8.1**	**0.012**	0.50 ± 0.03	0.62 ± 0.02	**−19.4**	**<0.001**
	K	3.88 ± 0.36	4.88 ± 0.76	**−20.5**	**<0.001**	5.18 ± 0.25	5.27 ± 0.35		0.536
	Ca	2.25 ± 0.23	2.07 ± 0.28		0.106	3.74 ± 0.24	4.14 ± 0.30	**−9.7**	**0.002**
	Mg	0.71 ± 0.04	0.78 ± 0.10	**−9.0**	**0.033**	0.48 ± 0.02	0.51 ± 0.02	**−5.9**	**<0.001**
Stem	C	38.32 ± 0.63	38.42 ± 0.41		0.621	39.43 ± 0.45	38.37 ± 0.43	**+2.8**	**<0.001**
	N	3.84 ± 0.30	3.60 ± 0.35		0.086	3.33 ± 0.19	3.57 ± 0.12	**−6.7**	**0.003**
	P	0.42 ± 0.03	0.49 ± 0.05	**−14.3**	**0.001**	0.46 ± 0.02	0.53 ± 0.02	**−13.2**	**<0.001**
	K	6.15 ± 0.51	6.83 ± 0.73	**−10.0**	**0.017**	5.31 ± 0.38	5.88 ± 0.25	**−9.7**	**<0.001**
	Ca	0.56 ± 0.08	0.60 ± 0.09		0.245	0.69 ± 0.05	0.78 ± 0.09	**−11.5**	**0.006**
	Mg	0.44 ± 0.05	0.43 ± 0.05		0.651	0.24 ± 0.02	0.26 ± 0.02	**−7.7**	**0.020**
Root	C	–	–	–	–	42.63 ± 1.22	43.31 ± 1.55		0.256
	N	–	–	–	–	3.96 ± 0.37	3.41 ± 0.34	**+16.1**	**0.001**
	P	–	–	–	–	0.41 ± 0.03	0.32 ± 0.04	**+28.1**	**<0.001**
	K	–	–	–	–	0.05 ± 0.02	0.04 ± 0.02		0.143
	Ca	–	–	–	–	1.01 ± 0.13	0.97 ± 0.15		0.463
	Mg	–	–	–	–	0.12 ± 0.01	0.12 ± 0.01		0.263

– Root samples were not analyzed due to mishandling.

4. Discussion

Chinese broccoli is fast-growing in hydroponic systems because fertigation can be optimized. It takes 50 to 60 days from sowing to harvest, depending on the ambient climatic conditions, such as air temperature and light. For example, low air temperature (15–20 °C) stimulated the bolting of flower stalks and shortened the time to harvest [28].

Here, Chinese broccoli grown in hydroponic culture was exposed to two different root temperatures (10 and 20 °C) in the last week before harvest. A low root temperature is widely known to limit shoot and root growth, and ultimately the biomass. For example, Poire et al. [29] reported that the leaf area and shoot fresh weight of *Ricinus communis* plants at lower root temperatures decreased throughout the experiment. In the experiment of *Agastache rugosa*, all plant growth parameters were restricted to cold root stress [13]. Plant growth of red leaf lettuce (*Lactuca sativa* L. cv. Red Wave) was decreased at a low root temperature (10 °C) as compared to temperatures of 20, 25 and 30 °C [10]. Similarly, we observed a reduction in the fresh weight of shoot and yield of Chinese broccoli. The decrease in fresh weight of shoot could be due to reduced water and nutrient uptake [30], a hormone signaling imbalance during the cooling treatment [31], or reduced photosynthesis [18,19]. However, shoot dry weight was not affected by root cooling, which indicated that the shoot water status was influenced by root temperature [32]. The balance between root water uptake and shoot transpiration determines the shoot's water status [33]. A higher transpiration rate was detected in the plants exposed to cooling temperatures in Exp-2 of the present study, but root water uptake (related to total root surface) was not examined. The reduction of shoot fresh biomass could be due to higher transpiration or combined effects with lower root water uptake. In the experiment of Poire et al. [29], the transpiration rate was statistically the same between the control and the cooling root group, but the values were even higher in the cooling plants. In addition, more negative xylem tension in the study of Poire et al. [29] confirmed that plants with cooled roots demand more water to enter the transpiration stream. Therefore, in our studies, increased water loss through transpiration could be a potential reason for the lower fresh weight, but an unaffected dry weight of the shoot. However, several studies suggest that stomatal conductance was reduced at lower root temperatures [33,34] and related this to the simultaneously

reduced root water uptake and subsequent carbon assimilation [35]. No consensus on root temperature effects has been achieved to date, possibly due to differences in species, treatment patterns, cultivation systems and study location [35]. Further studies on the regulatory mechanisms of transpiration and root water uptake are needed.

We aim to minimize the yield reduction under cold stress by reducing the root cooling treatment to one week before harvest. Several studies have investigated the effects of different magnitudes and durations of suboptimal root temperature on plant growth and development. For example, a long-term elevated root temperature has a more pronounced effect on the storage root biomass of sweet potato (*Ipomoea batatas*) than a short-term increase in root temperature [36]. Transient root cooling at 14 °C for two weeks increased the shoot dry weight of commercial pepper compared to constant root cooling over six weeks [37]. The long-term effects of low root temperature include cold acclimation of nutrient uptake, root respiration, photosynthesis and transpiration [38], therefore, the impact on biomass might be more complicated. In this study, the 18.9% reduction in yield was only observed in Exp-2 and lower than a previously documented 20.6% decrease in yield after long-term root cooling [14]. Therefore, shortening the duration of treatment alleviated the biomass reduction. Nevertheless, the effect was dependent on other environmental factors as well.

It has been widely demonstrated that photosynthesis is sensitive to temperature stress, and the impairment of photosynthesis apparatus is the first symptom of plants suffering temperature stress [30]. In the present study, we observed that the photosynthesis rates of the youngest mature leaf were not affected by root temperature. These results are in accordance with those of Kuwagata et al. [34] and Shimono et al. [39], who reported that the photosynthetic rate of rice was not affected by the low temperature of the nutrient solution. Moreover, Nagasuga et al. [33] attributed the decreased total dry weight of rice plants at lower root temperatures to reduced leaf area, while photosynthesis was not influenced. The transpiration and stomatal conductance at 47 DAS of Exp-2 were improved at the lower root temperature, which could be due to the alteration of the water status [32], while the lack of differences in these two parameters at 50 DAS between the two groups could be indicative of cold acclimation [38].

Soluble sugar levels determine the overall flavor and acceptance of vegetables by consumers. Glucose, fructose and sucrose are the major soluble sugars in Chinese broccoli [6]. The concentrations of three soluble sugars in stems were higher in comparison to leaves, consistent with previous studies [40]. Starch is the primary storage component [41], and its concentration was determined after soluble sugar extraction. It is possible that the distribution pattern of assimilated carbon into non-structural and structural components was altered at lower root temperatures [42]. The increase of carbohydrate concentration, especially in Exp-2, indicated that lower root temperatures increased non-structural carbon accumulation. Similarly, the concentration of carbohydrates in the leaves of *Ricinus communis* plants increased when roots were cooled [29]. In red leaf lettuce, a 7-day low root temperature treatment accelerated the accumulation of sugars [10]. These accumulated sugars may act as osmolytes to maintain turgor pressure, substrates for plants to survive stress and antioxidants to scavenge ROS [43]. Another explanation for the accumulated carbon is a cold girdling effect which reduces phloem solution flow to the roots and thus increases the shoot carbohydrate concentration [44]. Chadirin et al. [23] found that two weeks of root cooling at 5 °C increased the Brix value the same as one week of root cooling before harvest in spinach. Similarly, our results were consistent with the previous long-term root cooling, and the increase (%) of soluble sugars at root cooling was similar.

The increase of total chlorophyll concentration in the root cooling group of Exp-1 was consistent with our previous study [14]. In contrast, Adebooye et al. [45] noted a decrease of chlorophyll concentration of American snake tomato (*Trichosanthes cucumerina*) in response to root cooling from 30 to 20 °C. Anwar et al. [46] demonstrated that the chlorophyll content of cucumber seedlings was significantly reduced at a low root temperature (14 °C). Considering the warm conditions in Exp-1, which was conducted during summer, a lower root temperature could have relieved the negative effects of high

air temperature on photosystem II and Rubisco activity by showing an increase in total chlorophyll concentration. This explanation would be consistent with research about the manipulation of root temperature of lettuce aimed at effective production at high ambient temperatures [47]. Therefore, the impacts of root temperature on chlorophyll depend on other abiotic factors, the intensity of temperature stress and cultivars [46].

Chinese broccoli is considered a functional food that delivers high amounts of antioxidants, such as glucosinolates, which in plants can counteract the overproduction of ROS during abiotic or biotic stress [2]. Based on previous studies, the total glucosinolates of Chinese broccoli include aliphatic, indolic and aromatic groups [48,49]. However, no aromatic glucosinolates could be detected in our study. Rosa and Rodrigues [24] have documented variations in total and individual glucosinolates between different cultivars and individual parts of the same plant, which could explain the lack of aromatic glucosinolates in this study. Glucosinolate concentrations have also been reported to be affected by environmental factors, such as air temperature and light [24]. The production of glucosinolates is associated with antioxidant defense mechanisms against abiotic and biotic stress factors. Jasmonate and salicylic acid have been shown to be the two signaling molecules involved in the induction of different glucosinolates in plant defense, and the activation of various signal transduction pathways could alter levels of specific individual glucosinolates [12]. The results of previous studies that aimed to evaluate short-term and/or long-term effects of low air temperature stress on glucosinolates concentration are conflicting. Charron and Sams [50] and Steindal et al. [51] reported a higher concentration of total glucosinolates in leaves of broccoli and kale at lower ambient temperature, while, Rosa and Rodrigues [24] found no correlation between the air temperature and glucosinolate concentration of two-week-old cabbage seedlings (*Brassica. oleracea* var. *capitata*) grown at 20 and 30 °C for two days. Based on variable concentrations in different plant tissue and development stages, some studies concluded that the effects of air temperature stress depend on the plant organs, species, tested temperature range and other climatic factors [3,52,53].

The effects of root temperature stress on the concentration of glucosinolates have not yet been fully explored in the literature, especially with regard to long-term and short-term effects. After 48 h of root heating at 40 °C, aliphatic glucosinolate concentrations of wild rocket (*Diplotaxis tenuiifolia* cv *Frastagliata*) reduced, but aromatic and indolic glucosinolate levels were not affected [54]. Our previous study showed that long-term root cooling stress at 15 and 10 °C resulted in an increase of different individual as well as total glucosinolates in the leaves and stems of Chinese broccoli [14], especially indolic glucosinolates. However, research on *Arabidopsis thaliana* [55] and kale [56] concluded that aliphatic glucosinolates were more affected by temperature. Given the results of this study, the positive effects of short-term root cooling on aliphatic and indolic glucosinolates were similar, and these findings which are inconsistent results with previous research could be due to the different reactions of glucosinolates biosynthesis to air and root temperatures. Therefore, assessing the effects of root temperature on glucosinolates concentration is difficult due to the interference of confounding factors such as other climatic factors, plant developmental stage, plant organ and growing season [55]. As compared with the results of our long-term study, the impact of short-term root cooling (10 °C) was more pronounced, especially in Exp-2. Despite the reduced shoot fresh weight, the content of progoitrin and glucoraphanin increased in response to low root temperature. These results indicated that root cooling could be an effective method to improve the food quality of Chinese broccoli in terms of glucosinolates.

The element levels of Chinese broccoli were significantly affected by the nutrient solution temperature. The level of minerals in the shoot and roots can be associated with uptake rate and subsequent partitioning among different plant organs [45]. As a result of root cooling stress, hydraulic conductivity may be reduced, causing the uptake rate to be impeded [57]. In addition, minerals tend to be accumulated in the root for nutrient storage rather than translocated to other parts of the plants under stress conditions [58]. The increased fraction of N in roots and the reduced amount in the shoot at low root

temperature in the present study suggests this is the likely explanation. Increased carbon concentration in shoots with cooling roots in Exp-2 was likely caused by the slow sink metabolism and cold girding effect on the phloem pathway [29]. We also calculated elemental ratios (% based on DW) in Exp-2 to confirm the distribution of elements under the two root temperature treatments. The alteration of element allocation patterns was always associated with various external surrounding factors, such as temperature [59]. Aidoo et al. [60] subjected two cultivars of pepper to a low root zone temperature and found that more nitrogen was allocated to roots to ensure the survival of the whole plant; however, carbon allocation was not affected by low root temperature. In line with Aidoo et al. [60], our results in Exp-2 showed that nitrogen tended to accumulate in the roots, while other elements were unaffected.

5. Conclusions

The increasing demand for healthy vegetables by consumers has encouraged research on how to improve product quality. Our previous studies on the effects of long-term root cooling (10 °C) on Chinese broccoli indicated an improvement in the overall quality, but caused an unwanted reduction of yield. Therefore, here we shortened the root cooling treatment duration to one week before harvest and the reduction of yield was alleviated. No significant yield reduction was observed in Exp 1. Additionally, the food quality of Chinese broccoli was enhanced, exhibiting increased soluble sugar (glucose and fructose) concentration in the leaves and several individual glucosinolates in stems and leaves. Furthermore, shortening the treatment duration is more economical, reducing energy cost associated with root cooling. We conclude that a short period of root cooling before harvest is a promising option to improve the quality of Chinese broccoli yet maintain yield. We also found that the effect of root cooling interacts with other climatic factors, such as ambient temperature and light intensity. Further studies are needed to clarify the interaction between these climatic factors to optimize the yield and food quality.

References

1. Guo, J.-J.; Tan, X.; Fu, H.-L.; Chen, J.-X.; Lin, X.-X.; Ma, Y.; Yang, Z.-Y. Selection for Cd pollution-safe cultivars of Chinese kale (*Brassica alboglabra* LH Bailey) and biochemical mechanisms of the cultivar-dependent Cd accumulation involving in Cd subcellular distribution. *J. Agric. Food Chem.* **2018**, *66*, 1923–1934. [CrossRef] [PubMed]
2. Sun, B.; Yan, H.; Zhang, F.; Wang, Q. Effects of plant hormones on main health-promoting compounds and antioxidant capacity of Chinese kale. *Food Res. Int.* **2012**, *48*, 359–366. [CrossRef]

3.   Martínez-Ballesta, M.D.C.; Moreno, D.A.; Carvajal, M. The physiological importance of glucosinolates on plant response to abiotic stress in Brassica. *Int. J. Mol. Sci.* **2013**, *14*, 11607. [CrossRef] [PubMed]

4.   Fahey, J.W.; Wehage, S.L.; Holtzclaw, W.D.; Kensler, T.W.; Egner, P.A.; Shapiro, T.A.; Talalay, P. Protection of humans by plant glucosinolates: Efficiency of conversion of glucosinolates to isothiocyanates by the gastrointestinal microflora. *Cancer Prev. Res.* **2012**, *5*, 603–611. [CrossRef] [PubMed]

5.   Dinkova-Kostova, A.T.; Kostov, R.V. Glucosinolates and isothiocyanates in health and disease. *Trends Mol. Med.* **2012**, *18*, 337–347. [CrossRef] [PubMed]

6.   Rosa, E.; David, M.; Gomes, M.H. Glucose, fructose and sucrose content in broccoli, white cabbage and Portuguese cabbage grown in early and late seasons. *J. Sci. Food Agric.* **2001**, *81*, 1145–1149. [CrossRef]

7.   Kawashima, L.M.; Valente Soares, L.M. Mineral profile of raw and cooked leafy vegetables consumed in Southern Brazil. *J. Food Compost. Anal.* **2003**, *16*, 605–611. [CrossRef]

8.   Islam, M.Z.; Lee, Y.-T.; Mele, M.A.; Choi, I.-L.; Kang, H.-M. The effect of phosphorus and root zone temperature on anthocyanin of red romaine lettuce. *Agronomy* **2019**, *9*, 47. [CrossRef]

9.   Sakamoto, M.; Suzuki, T. Elevated root-zone temperature modulates growth and quality of hydroponically grown carrots. *Agric. Sci.* **2015**, *6*, 749–757. [CrossRef]

10.   Sakamoto, M.; Suzuki, T. Effect of root-zone temperature on growth and quality of hydroponically grown red leaf lettuce (*Lactuca sativa* L. cv. Red Wave). *Am. J. Plant Sci.* **2015**, *6*, 2350. [CrossRef]

11.   Ito, A.; Shimizu, H. Effect of temperature and duration of root chilling on the balance between antioxidant activity and oxidative stress in spinach. *Environ. Control. Biol.* **2020**, *58*, 115–121. [CrossRef]

12.   Variyar, P.S.; Banerjee, A.; Akkarakaran, J.J.; Suprasanna, P. Role of glucosinolates in plant stress tolerance. In *Emerging Technologies and Management of Crop Stress Tolerance*; Ahmad, P., Rasool, S., Eds.; Academic Press: San Diego, CA, USA, 2014; pp. 271–291.

13.   Lam, V.P.; Kim, S.J.; Bok, G.J.; Lee, J.W.; Park, J.S. The effects of root temperature on growth, physiology, and accumulation of bioactive compounds of *Agastache rugosa*. *Agriculture* **2020**, *10*, 162. [CrossRef]

14.   He, F.; Thiele, B.; Santhiraraja-Abresch, S.; Watt, M.; Kraska, T.; Ulbrich, A.; Kuhn, A.J. Effects of root temperature on the plant growth and food quality of Chinese broccoli (*Brassica oleracea* var. *alboglabra* Bailey). *Agronomy* **2020**, *10*, 702. [CrossRef]

15.   He, F.; Thiele, B.; Watt, M.; Kraska, T.; Ulbrich, A.; Kuhn, A.J. Effects of root cooling on plant growth and fruit quality of cocktail tomato during two consecutive seasons. *J. Food Qual.* **2019**, *2019*. [CrossRef]

16.   Hwang, I.; Manoharan, R.K.; Kang, J.-G.; Chung, M.-Y.; Kim, Y.-W.; Nou, I.-S. Genome-wide identification and characterization of bzip transcription factors in *Brassica oleracea* under Cold Stress. *Biomed. Res. Int.* **2016**, *2016*, 4376598. [CrossRef]

17.   Kayum, M.A.; Kim, H.-T.; Nath, U.K.; Park, J.-I.; Kho, K.H.; Cho, Y.-G.; Nou, I.-S. Research on biotic and abiotic stress related genes exploration and prediction in *Brassica rapa* and *B. oleracea*: A review. *Plant Breed. Biotechnol.* **2016**, *4*, 135–144. [CrossRef]

18.   Aroca, R.; Tognoni, F.; Irigoyen, J.J.; Sánchez-Díaz, M.; Pardossi, A. Different root low temperature response of two maize genotypes differing in chilling sensitivity. *Plant Physiol. Biochem.* **2001**, *39*, 1067–1073. [CrossRef]

19.   Schwarz, D.; Rouphael, Y.; Colla, G.; Venema, J.H. Grafting as a tool to improve tolerance of vegetables to abiotic stresses: Thermal stress, water stress and organic pollutants. *Sci. Hortic.* **2010**, *127*, 162–171. [CrossRef]

20.   Eremina, M.; Rozhon, W.; Poppenberger, B. Hormonal control of cold stress responses in plants. *Cell. Mol. Life Sci.* **2016**, *73*, 797–810. [CrossRef] [PubMed]

21.   Ramakrishna, A.; Ravishankar, G.A. Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signal. Behav.* **2011**, *6*, 1720–1731. [CrossRef] [PubMed]

22.   Ogawa, E.; Hikosaka, S.; Goto, E. Effects of nutrient solution temperature on the concentration of major bioactive compounds in red perilla. *J. Agric. Meteorol.* **2018**, *74*, 71–78. [CrossRef]

23.   Chadirin, Y.; Hidaka, K.; Takahashi, T.; Sago, Y.; Wajima, T.; Kitano, M. Application of temperature stress to roots of spinach I. Effect of the low temperature stress on quality. *Environ. Control. Biol.* **2011**, *49*, 133–139. [CrossRef]

24.   Rosa, E.A.S.; Rodrigues, P.M.F. The effect of light and temperature on glucosinolate concentration in the leaves and roots of cabbage seedlings. *J. Sci. Food Agric.* **1998**, *78*, 208–212. [CrossRef]

25.   Füllner, K.; Temperton, V.M.; Rascher, U.; Jahnke, S.; Rist, R.; Schurr, U.; Kuhn, A.J. Vertical gradient in soil temperature stimulates development and increases biomass accumulation in barley. *Plant Cell Environ.* **2012**, *35*, 884–892. [CrossRef] [PubMed]

26.   Sun, B.; Yan, H.; Liu, N.; Wei, J.; Wang, Q. Effect of 1-MCP treatment on postharvest quality characters, antioxidants and glucosinolates of Chinese kale. *Food Chem.* **2012**, *131*, 519–526. [CrossRef]

27.   Viola, R.; Davies, H.V. A microplate reader assay for rapid enzymatic quantification of sugars in potato tubers. *Potato Res.* **1992**, *35*, 55–58. [CrossRef]

28.   Xian, Y.; Yunying, Y. The effects of temperature on flower bud differentiation, yield and quality formation in Chinese kale (*Brassica alboglabra* Bailey). *J. South China Agric. Univ.* **2002**, *23*, 5–7. [CrossRef]

29.   Poiré, R.; Schneider, H.; Thorpe, M.R.; Kuhn, A.J.; Schurr, U.; Walter, A. Root cooling strongly affects diel leaf growth dynamics, water and carbohydrate relations in *Ricinus communis*. *Plant Cell Environ.* **2010**, *33*, 408–417. [CrossRef] [PubMed]

30.   Hao, H.-P.; Jiang, C.-D.; Zhang, S.-R.; Tang, Y.-D.; Shi, L. Enhanced thermal-tolerance of photosystem II by elevating root zone temperature in *Prunus mira Koehne* seedlings. *Plant Soil* **2012**, *353*, 367–378. [CrossRef]

31. Aloni, B.; Cohen, R.; Karni, L.; Aktas, H.; Edelstein, M. Hormonal signaling in rootstock–scion interactions. *Sci. Hortic.* **2010**, *127*, 119–126. [CrossRef]

32. Dodd, I.C.; He, J.; Turnbull, C.G.; Lee, S.K.; Critchley, C. The influence of supra-optimal root-zone temperatures on growth and stomatal conductance in *Capsicum annuum* L. *J. Exp. Bot.* **2000**, *51*, 239–248. [CrossRef] [PubMed]

33. Nagasuga, K.; Murai-Hatano, M.; Kuwagata, T. Effects of low root temperature on dry matter production and root water uptake in rice plants. *Plant Prod. Sci.* **2011**, *14*, 22–29. [CrossRef]

34. Kuwagata, T.; Ishikawa-Sakurai, J.; Hayashi, H.; Nagasuga, K.; Fukushi, K.; Ahamed, A.; Takasugi, K.; Katsuhara, M.; Murai-Hatano, M. Influence of low air humidity and low root temperature on water uptake, growth and aquaporin expression in rice plants. *Plant Cell Physiol.* **2012**, *53*, 1418–1431. [CrossRef] [PubMed]

35. Wang, X.; Zhang, W.; Miao, Y.; Gao, L. Root-zone warming differently benefits mature and newly unfolded leaves of *Cucumis sativus* l. seedlings under sub-optimal temperature stress. *PLoS ONE* **2016**, *11*, e0155298. [CrossRef]

36. Taranet, P.; Kirchhof, G.; Fujinuma, R.; Menzies, N. Root zone temperature alters storage root formation and growth of sweetpotato. *J. Agron. Crop Sci.* **2018**, *204*, 313–324. [CrossRef]

37. Aidoo, M.K.; Sherman, T.; Lazarovitch, N.; Fait, A.; Rachmilevitch, S. Physiology and metabolism of grafted bell pepper in response to low root-zone temperature. *Funct. Plant Biol.* **2019**, *46*, 339–349. [CrossRef] [PubMed]

38. Atkin, O.K.; Edwards, E.J.; Loveys, B.R. Response of root respiration to changes in temperature and its relevance to global warming: REVIEW Temperature and root respiration. *New Phytol.* **2000**, *147*, 141–154. [CrossRef]

39. Shimono, H.; Hasegawa, T.; Fujimura, S.; Iwama, K. Responses of leaf photosynthesis and plant water status in rice to low water temperature at different growth stages. *Field Crops Res.* **2004**, *89*, 71–83. [CrossRef]

40. Wang, Y.-Q.; Hu, L.-P.; Liu, G.-M.; Zhang, D.-S.; He, H.-J. Evaluation of the nutritional quality of Chinese kale (*Brassica alboglabra* Bailey) using UHPLC-Quadrupole-Orbitrap MS/MS-based metabolomics. *Molecules* **2017**, *22*. [CrossRef]

41. Liu, W.; Su, J.; Li, S.; Lang, X.; Huang, X. Non-structural carbohydrates regulated by season and species in the subtropical monsoon broad-leaved evergreen forest of Yunnan Province, China. *Sci. Rep.* **2018**, *8*, 1083. [CrossRef] [PubMed]

42. Arai-Sanoh, Y.; Ishimaru, T.; Ohsumi, A.; Kondo, M. Effects of soil temperature on growth and root function in rice. *Plant Prod. Sci.* **2010**, *13*, 235–242. [CrossRef]

43. Sami, F.; Yusuf, M.; Faizan, M.; Faraz, A.; Hayat, S. Role of sugars under abiotic stress. *Plant Physiol. Biochem.* **2016**, *109*, 54–61. [CrossRef] [PubMed]

44. Peuke, A.D.; Windt, C.; Van As, H. Effects of cold-girdling on flows in the transport phloem in *Ricinus communis*: Is mass flow inhibited? *Plant Cell Environ.* **2006**, *29*, 15–25. [CrossRef] [PubMed]

45. Adebooye, O.C.; Schmitz-Eiberger, M.; Lankes, C.; Noga, G.J. Inhibitory effects of sub-optimal root zone temperature on leaf bioactive components, photosystem II (PS II) and minerals uptake in *Trichosanthes cucumerina* L. Cucurbitaceae. *Acta Physiol. Plant.* **2010**, *32*, 67–73. [CrossRef]

46. Anwar, A.; Di, Q.; Yan, Y.; He, C.; Li, Y.; Yu, X. Exogenous 24-epibrassinolide alleviates the detrimental effects of suboptimal root zone temperature in cucumber seedlings. *Arch. Agron. Soil Sci.* **2019**, *65*, 1927–1940. [CrossRef]

47. Bumgarner, N.R.; Scheerens, J.C.; Mullen, R.W.; Bennett, M.A.; Ling, P.P.; Kleinhenz, M.D. Root-zone temperature and nitrogen affect the yield and secondary metabolite concentration of fall- and spring-grown, high-density leaf lettuce. *J. Sci. Food Agric.* **2012**, *92*, 116–124. [CrossRef] [PubMed]

48. La, G.-X.; Fang, P.; Li, Y.-J.; Wang, Y. Determination of desulpho-glucosinolates in bolting stems of Chinese kale by liquid chromatography-mass spectrometry. *J. Zhejiang Univ.* **2008**, *34*. [CrossRef]

49. La, G.-X.; Fang, P.; Teng, Y.-B.; Li, Y.-J.; Lin, X.-Y. Effect of CO₂ enrichment on the glucosinolate contents under different nitrogen levels in bolting stem of Chinese kale (*Brassica alboglabra* L.). *J. Zhejiang Univ. Sci. B* **2009**, *10*, 454–464. [CrossRef]

50. Charron, C.S.; Sams, C.E. Glucosinolate content and myrosinase activity in rapid-cycling *Brassica oleracea* grown in a controlled environment. *J. Am. Soc. Hortic. Sci.* **2004**, *129*, 321–330. [CrossRef]

51. Steindal, A.L.H.; Mølmann, J.; Bengtsson, G.B.; Johansen, T.J. Influence of day length and temperature on the content of health-related compounds in broccoli (*Brassica oleracea* L. var. *italica*). *J. Agric. Food Chem.* **2013**, *61*, 10779–10786. [CrossRef]

52. Engelen-Eigles, G.; Holden, G.; Cohen, J.D.; Gardner, G. The effect of temperature, photoperiod, and light quality on gluconasturtiin concentration in watercress (*Nasturtium officinale* R. Br.). *J. Agric. Food Chem.* **2006**, *54*, 328–334. [CrossRef]

53. Farnham, M.W.; Wilson, P.E.; Stephenson, K.K.; Fahey, J.W. Genetic and environmental effects on glucosinolate content and chemoprotective potency of broccoli. *Plant Breed.* **2004**, *123*, 60–65. [CrossRef]

54. Cocetta, G.; Mishra, S.; Raffaelli, A.; Ferrante, A. Effect of heat root stress and high salinity on glucosinolates metabolism in wild rocket. *J. Plant Physiol.* **2018**, *231*, 261–270. [CrossRef]

55. Kissen, R.; Eberl, F.; Winge, P.; Uleberg, E.; Martinussen, I.; Bones, A.M. Effect of growth temperature on glucosinolate profiles in *Arabidopsis thaliana* accessions. *Phytochemistry* **2016**, *130*, 106–118. [CrossRef] [PubMed]

56. Steindal, A.L.H.; Rødven, R.; Hansen, E.; Mølmann, J. Effects of photoperiod, growth temperature and cold acclimatisation on glucosinolates, sugars and fatty acids in kale. *Food Chem.* **2015**, *174*, 44–51. [CrossRef]

57. Baghour, M.; Ragala, L.; Moreno, D.A.; Villora, G.; Hernández, J.; Castilla, N.; Romero, L. Effect of root zone temperature on accumulation of molybdenum and nitrogen metabolism in potato plants. *J. Plant Nutr.* **2003**, *26*, 443–461. [CrossRef]

58. Pettersson, S. Low root zone temperature effects on net mineral nutrient uptake and distribution in barley (*Hordeum vulgare*). *J. Plant Physiol.* **1995**, *145*, 459–464. [CrossRef]
59. Starck, Z.; Niemyska, B.; Bogdan, J.; Tawalbeh, R.N.A. Response of tomato plants to chilling stress in association with nutrient or phosphorus starvation. *Plant Soil* **2000**, *226*, 99–106. [CrossRef]
60. Aidoo, M.K.; Sherman, T.; Lazarovitch, N.; Fait, A.; Rachmilevitch, S. A bell pepper cultivar tolerant to chilling enhanced nitrogen allocation and stress-related metabolite accumulation in the roots in response to low root-zone temperature. *Physiol. Plant.* **2017**, *161*, 196–210. [CrossRef] [PubMed]