



## Article

# Effects of Root Temperature on the Plant Growth and Food Quality of Chinese Broccoli (*Brassica oleracea* var. *alboglabra* Bailey)

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**Abstract:** Root temperature has long been considered an essential environmental factor influencing the plant's physiology. However, little is known about the effect of root temperature on the quality of the food produced by the plant, especially that of horticultural crops. To fill this gap, two independent root cooling experiments (15 °C vs. 20 °C and 10 °C vs. 20 °C) were conducted in autumn 2017 and spring 2018 in hydroponics with Chinese broccoli (*Brassica oleracea* var. *alboglabra* Bailey) under greenhouse conditions. The aim was to investigate the effect of root temperature on plant growth (biomass, height, yield) and food quality (soluble sugars, total chlorophyll, starch, minerals, glucosinolates). A negative impact on shoot growth parameters (yield, shoot biomass) was detected by lowering the root temperature to 10 °C. Chinese broccoli showed no response to 15 °C root temperature, except for an increase in root biomass. Low root temperature was in general associated with a higher concentration of soluble sugars and total chlorophyll, but lower mineral levels in stems and leaves. Ten individual glucosinolates were identified in the stems and leaves, including six aliphatic and four indolic glucosinolates. Increased levels of neoglucobrassicin in leaves tracked root cooling more closely in both experiments. Reduction of root temperature by cooling could be a potential method to improve certain quality characters of Chinese broccoli, including sugar and glucosinolate levels, although at the expense of shoot biomass.

**Keywords:** Chinese broccoli (*Brassica oleracea* var. *alboglabra* Bailey); root temperature; glucosinolates; soluble sugars; chlorophyll; starch; minerals

## 1. Introduction

Over the last decades, research has shifted towards investigating the essential minerals and health-promoting phytochemicals of fruit and vegetables and their significance for human nutrition. Consumers increasingly demand healthy, attractive and tasty horticultural products. Balancing yield and quality in horticultural crop production requires increased research, especially into the effects of cultivation factors and environment. Root temperature, one important component of cultivation microclimate, has an important role in a variety of structural and functional characteristics of plants [1].

In hydroponic systems, root temperature is generally different from ambient temperature [2] but is convenient and economical to manage and control. Most studies about root temperature concentrate on physiological impacts on plants, such as water and nutrient uptake [3,4], photosynthesis and transpiration [5,6]. Information on the effects of root temperature on the quality (organic acids, soluble sugars, minerals and antioxidants) of horticultural crops is incomplete and often contradictory. For example, by lowering the root temperature, some authors found positive effects on food quality of cucumber seedlings, carrots and red leaf lettuce [7–9], while still other works found the quality of strawberries unaffected by root temperature [10].

Vegetables in the Brassica genus are consumed around the world and are important for human and animal health [11]. Chinese broccoli belongs to the same species *Brassica oleracea* (common broccoli) but in the cultivar group *alboglabra* [12]. It is also known as Chinese kale, Kailan, or Gailan. Chinese broccoli is mostly consumed at the bolting stem stage and is one of the most popular leaf vegetables in South China and Southeast Asia [13]. Its abundance in health-promoting antioxidants and essential minerals [14] resulted in greater Chinese broccoli consumption in Europe and America in recent years [15].

Glucosinolates are the prominent antioxidant existing in *Brassica* spp., and recent evidence suggests glucosinolates in diets may prevent biological activities associated with oxidative stress, inflammation and cancer [16]. The pungent flavor and bitter taste of *Brassica* vegetables are associated with the breakdown products of glucosinolates isothiocyanates [17]. Sugar levels, together with glucosinolates, also influence the flavor and acceptance by the consumer [11]. Chinese broccoli, like other leaf vegetables, is believed to provide a modest source of essential minerals such as K, Ca and Mg, in well-balanced diets [18]. Air temperature has been reported to influence the levels of glucosinolates and sugars in curly kale and turnips [19,20]. Previous studies have also been conducted into the effects of rooting environment temperature on certain Brassica vegetables [4,21–24]. However, these studies mainly focus on growth and biomass. For instance, in broccoli, the yield was increased with a root temperature above 21 °C to 25 °C but slightly affected by a root temperature lower than 21 °C [4]. So far, we are unaware of published studies of both the growth and quality of Chinese broccoli in response to root temperature.

Understanding how root temperature affects vegetative development of Chinese broccoli is important for the optimization of management strategies to achieve better quality. Hence, this study was conducted to analyze changes in biomass and nutrient composition in *Brassica oleracea* var. *alboglabra* cv “Cuimei” grown in hydroponics under different root temperatures. The hypothesis was that manipulation of root temperature by cooling down would result in higher accumulation of nutritionally important phytochemicals (glucosinolates, total chlorophyll, soluble sugars) and essential minerals (N, P, K, Ca, Mg) of Chinese broccoli without reducing the yield.

## 2. Materials and Methods

### 2.1. Plant Materials and Growth Conditions

The seeds of Chinese broccoli (*Brassica oleracea* var. *alboglabra* cv “Cuimei”) were obtained from the Guangdong Academy of Agricultural Sciences (Guangdong, China). The seeds were first sterilized with hot water (50–55 °C) for 10 min [13] and then sown on paper maintained with distilled water spray until seedling emergence. The air temperature was kept at 25/20 °C (day/night) and light was avoided. After germination, young seedlings with cotyledon and the first true leaf were transferred to small pots with sand, and irrigated with distilled water in the first week and then with 25% strength Hoagland solution containing (1.25 mM CaNO<sub>3</sub>, 1.25 mM KNO<sub>3</sub>, 0.5 mM MgSO<sub>4</sub>, 0.25 mM KH<sub>2</sub>PO<sub>4</sub>, 22.4 µM Fe(EDTA), 2.5 µM MnCl<sub>2</sub>, 0.25 µM CuSO<sub>4</sub>, 0.25 µM ZnSO<sub>4</sub>, 12.5 µM H<sub>3</sub>BO<sub>3</sub>, 0.125 µM Na<sub>2</sub>MoO<sub>4</sub>). After the emergence of the third true leaf (31 and 33 DAS in Exp-1 and Exp-2 respectively), 32 seedlings of uniform size were transplanted to eight containers (28 × 43 × 17 cm) with nutrient solution, each holding four plants. The nutrient solution was aerated with aquarium air pumps

connected with an airstone, which gradually diffuses air into the tank through transparent tubes. The nutrient solution was 50% strength Hoagland solution (2.5 mM CaNO<sub>3</sub>, 2.5 mM KNO<sub>3</sub>, 1 mM MgSO<sub>4</sub>, 0.5 mM KH<sub>2</sub>PO<sub>4</sub>, 44.8 µM Fe(EDTA), 5 µM MnCl<sub>2</sub>, 0.5 µM CuSO<sub>4</sub>, 0.5 µM ZnSO<sub>4</sub>, 25 µM H<sub>3</sub>BO<sub>3</sub>, 0.25 µM Na<sub>2</sub>MoO<sub>4</sub>) and was changed every week to guarantee a constant pH (6.0–6.5) and EC value (1.1–1.2 ms/cm). The experiment was carried out under greenhouse conditions with a daily 16-h light period and relative air humidity of around 50%. Light and air temperature of the growth condition were recorded by the climate station within the greenhouse.

## 2.2. Root Temperature Setup

Two experiments were carried out under greenhouse conditions (Forschungszentrum Jülich, Germany) from 2017 to 2018 as shown in Table 1. Eight containers were arranged in two rows, and each row was treated with one root temperature. Four containers of each group were connected with pipes (Ø 11.5 cm) to guarantee uniform root temperature within the group. Root temperature (Table 1) was kept constant throughout the treatment period by circulating the solution through a thermostat (Oceanrunner OR1200, Aqua Medic, Germany). Root temperature treatment was initiated 3 days after transplanting in the same hydroponic system at the fourth true leaf stage (34 and 36 DAS).

**Table 1.** Overview of the setup for growth experiments of Chinese broccoli described in this study, indicating the plant date, root temperature, harvest date and treatment duration in the two experiments.

Experiments	Date	Treatment		
		Root Temperature	Harvest	Duration
Exp-1	20 Aug–11 Oct, 2017	15 vs. 20 °C	11 October, 2017 29 Mar	24 Sep–11 Oct, 2017
Exp-2	13 Feb–12 Apr, 2018	10 vs. 20 °C	5 Apr 12 Apr, 2018	21 Mar–12 Apr, 2018

## 2.3. Harvest and Sample Preparation

In Exp-1, all 32 plants were harvested when 80% had reached marketable maturity (the height of the stem is the same as that of the leaves) [25]. Each plant was a replicate. After harvest, shoot height was measured from cotyledon scar to base of petiole on youngest fully developed leaf and root length was measured from cotyledon scar to the farthest point of the root. Plants were first separated into shoots and roots and fresh weight (FW) of shoot and root were weighed and recorded. From the 5th node above, the upper tender plant parts, comprising the main stem together with terminal floral buds and 6–7 leaves, were regarded as consumable and recorded as marketable yield [25]. The edible parts were separated into stem and leaves. Stem and leaves were cut and further divided into two parts. One part was frozen on site with liquid nitrogen immediately and stored at −80 °C for further biochemical analysis. Another part was dried at 65 °C until constant weight for dry weight and element analysis. Root dry weight (DW) was weighed and recorded directly. Shoot dry weight (DW) was calculated based on the ratio before and after drying of the sampled stems and leaves. Shoot ratio is calculated with the below formula,

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

The frozen samples were ground with a mortar and pestle with liquid nitrogen and then stored at −80 °C. The dried samples were ground in a mixer mill (MM400, Retsch, Haan, Germany).

In Exp-2, five plants of each group were harvested every seven days, three times after the initiation of the root temperature treatment. For the first two harvests (43 and 50 DAS), each young plant was sampled in order to measure the shoot FW, root FW, shoot height and root length. Leaves and stems were separated into two parts for freezing and drying. The sampling procedure of the final harvest (57 DAS) was the same as in Exp-1.

#### 2.4. Elemental Analysis

Ground dried samples were digested in HNO<sub>3</sub>, H<sub>2</sub>O<sub>2</sub> and HF (hydrogen fluoride) by microwave and then analyzed by ICP-OES (Inductively Coupled Plasma with Optical Emission Spectroscopy, Elan 6000, Perkin Elmer, Sciex; Agilent 7500ce, Planitz, Germany) for the determination of C, N, P, K, Ca and Mg, as described by He et al. [26].

#### 2.5. Soluble Sugars, Total Chlorophyll and Starch Quantification

The extraction of soluble sugars, total chlorophyll and starch was conducted with the same procedure. Completely ground samples (50 mg) were homogenized with 400 µL 80% ethanol at 80 °C for 15 min and then centrifuged at 13,200 rpm for 3 min. The supernatant was removed and the sample pellets were resuspended in 400 µL 50% ethanol and extracted again with the same procedure. The step was repeated twice with 200 µL of 80% ethanol until the pellets were colorless. Supernatant was pooled together and 2 mL was used for soluble sugar and total chlorophyll assays. During the extraction, light was avoided and the supernatant was kept on ice.

Total chlorophyll concentration was measured directly after extraction. A 400 µL aliquot of supernatant was diluted with 600 µL of 80% ethanol and measured at 652 nm with a microplate reader (Synergy™ 2 Multi-Mode, BioTek, Winooski, Vermont, USA).

Soluble sugars concentrations were determined by enzymatic analysis based on Viola & Davies [27] with minor adjustments. A 20 µL aliquot of supernatant was added in a microplate well with 15 µmol imidazole buffer (Merck, Darmstadt, Germany), 162 µg NADP (Roche diagnostics, Roche Holding AG, Basel, Switzerland), 260 µg ATP (Merck, Darmstadt, Germany) and 2 µL activated glucose-6-phosphate dehydrogenase (Roche diagnostics, Roche Holding AG, Basel, Switzerland) and then the absorbance was recorded at 340 nm by microplate reader as the baseline (A1). An aliquot of 2 µL of activated hexokinase (Roche diagnostics, Roche Holding AG, Basel, Switzerland) was added to the well and the absorbance was measured again at 340 nm until the reaction was complete at room temperature (A2). The concentration of glucose was calculated based on the difference between A2 and A1 due to the conversion of NAD to NADP by the reaction of glucose to 6-phosphogluconate. Afterward, 2 µL activated phosphoglucose-isomerase (Roche diagnostics, Roche Holding AG, Basel, Switzerland) and 2 µL activated invertase (Roche diagnostics, Roche Holding AG, Basel, Switzerland) were added successively, and the same procedure was repeated to record the absorbance (A3) and (A4) respectively. The concentrations of fructose and sucrose were based on the difference between A3 and A2 and between A4 and A3, respectively.

Starch assays were performed on the pellets from previous extractions of soluble sugars and chlorophyll. After the last extraction, the pellets were washed with distilled water and dried overnight. Then 500 µL distilled water was added to the pellets and autoclaved for 90 min to disperse the starch. The concentration of starch was calculated based on the quantity of glucose after conversion of starch to glucose under the effect of amyloglucosidase and α-amylase (Roche diagnostics, Roche Holding AG, Basel, Switzerland). A 100 µL aliquot autoclaved sample was incubated with 400 µL incubation buffer containing 15 µmol sodium acetate, 16 µL amyloglucosidase and 0.16 µL α-amylase at 37 °C for 16 h for the conversion of starch to glucose. Analysis of starch was the same as the procedure of glucose, but with a slight difference in buffer composition: 15 µmol tris, 1.5 µmol Mg<sup>2+</sup>, 162 µg NADP, 260 µg ATP, 2 µL activated glucose-6-phosphate dehydrogenase. The concentration of starch was calculated from the difference between the absorbance before and after the reaction.

#### 2.6. Glucosinolates Analysis

The extraction of intact glucosinolates from stems and leaves was performed according to the method of Volden et al. [28] and Doheny-Adams et al. [29] with some modifications. Approximately 60 mg of frozen sample powder were extracted in 1.5 mL of 80% (v/v) methanol and held at room temperature for 30 min. The sample was then homogenized for another 30 min and centrifuged at

13,200 rpm for 15 min. The supernatants were collected, and the pellets were resuspended with 1.5 mL of 80% methanol and centrifuged. The pooled supernatants were evaporated to dryness at room temperature in a vacuum-evaporator (Eppendorf Concentrator 5301, Hamburg, Germany) for around 4 h and re-dissolved in 240 µL of 50% (v/v) methanol. Prior to LC-MS analysis, all samples were filtered through 0.2 µm filters (Whatman, PTFE, 4 mm, Dassel, Germany).

Quantification of glucosinolates was carried out using a 1260 Infinity Agilent HPLC system (degasser, binary pump, autosampler, thermostatic column compartment) coupled to an Agilent triple-quadrupole mass spectrometry system 6420 (Agilent, Agilent Co., Santa Clara, California, USA). Separation of glucosinolates was achieved on a Nucleodur C18 Gravity-SB column (150 × 3 mm, 3 µm; Macherey-Nagel, Düren, Germany). A precolumn as the protection and first filter was used. The mobile phase was a mixture of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B) running at a flow rate of 1 mL min<sup>-1</sup>. The gradient program was 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. The electrospray ionization (ESI) interface of the mass spectrometer was driven in the negative mode. The capillary voltage was set to 5500 V. The gas temperature and gas flow of nitrogen was 300 °C and 10 L min<sup>-1</sup>, respectively. The nebulizer pressure was set to 60 psi. Mass spectrometric detection in the MRM mode was applied for the quantification of glucosinolates (Table 2).

**Table 2.** MRM parameter for glucosinolates used for quantification and compound confirmation.

	Compound	Precursor Ion [m/z]	Product Ion [m/z]	Cone Voltage [V]	Collision Energy [V]
<b>Aliphatic</b>	Sinigrin	358.1	195	100	20
	Progoitrin	388	195	100	20
	Glucoraphanin	436.1	372.1	100	22
	Gluconapin	372.1	359	100	20
	Glucoiberin	422.1	358.1	100	22
	Glucoalyssin	450.2	386.2	100	22
<b>Indolic</b>	Glucobrassicin	447.1	259.1	100	22
	4-Methoxyglucobrassicin	477.1	195	100	23
	Neoglucobrassicin	477.1	446.1	100	13
	4-Hydroxyglucobrassicin	463.1	267	100	18

The concentration of each glucosinolate was determined by external calibration using a standard solution composed of pure standards compounds (Phytoflan Diehm & Neuberger GmbH, Heidelberg, Germany). Each sample was extracted and analyzed in duplicate and the results are reported as µmol per 100 g fresh weight (FW).

### 2.7. Statistical Analysis

All statistical analyses were performed using R version 3.1.3 and R studio version. Each plant was regarded as one biological replicate. Considering the different environmental conditions in each experiment, data from each experiment were analyzed separately. Experimental results were expressed as the means ± standard deviation. Differences due to root temperature treatment were determined by the least significant differences at  $\alpha = 0.05$ . The student *t*-test was used to make pair-wise comparisons between treatments and determine the probability of statistical difference.

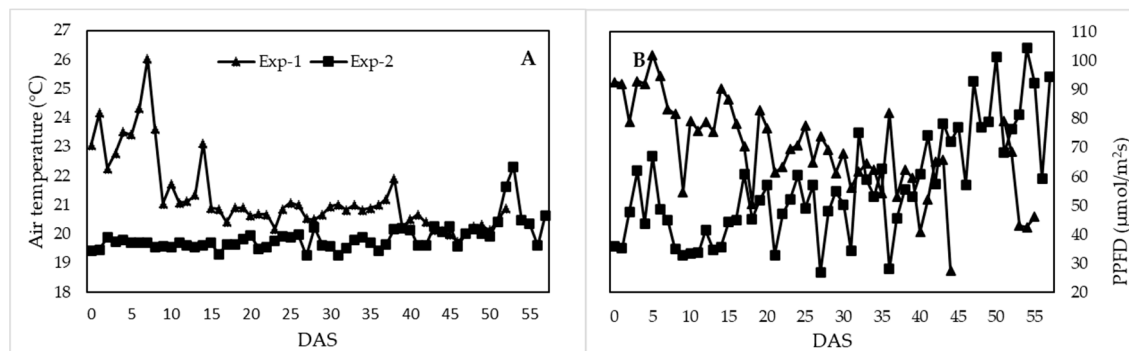
## 3. Results

### 3.1. Greenhouse Climate Conditions

The experiments were conducted in the autumn of 2017 and spring of 2018 in a glasshouse, and the climate conditions were affected by outdoor light and air temperature (Figure 1). The fluctuations of



air temperature and light were consistent across experiments but average daily air temperature and light of Exp-2 were lower than those of Exp-1, especially in the first 30 days after sowing.



**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.

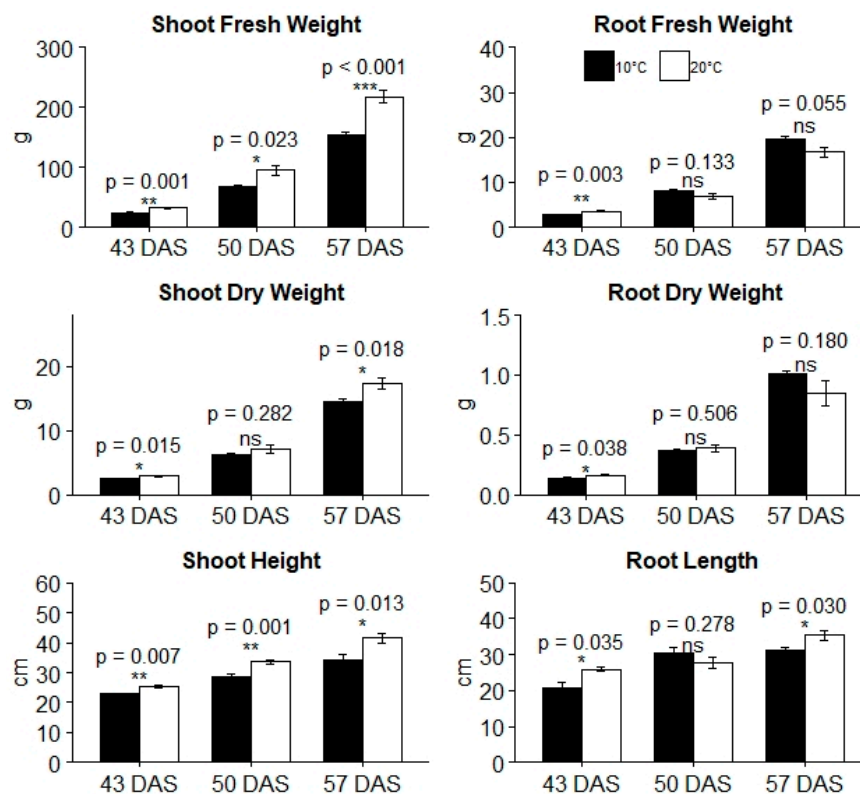
### 3.2. Effects of Root Temperature on Plant Growth

In Exp-1, we did not detect visual differences in the plant morphology and growth at two root temperatures 15 °C and 20 °C. In addition, stem bolting was also not affected by root temperatures. Based on the results (Table 3), no significant root temperature effect ( $p > 0.05$ ) on neither shoot biomass (FW and DW) nor shoot height further was revealed. However, at 15 °C root temperature, root FW and DW increased by 35.8% and 23.1% respectively in comparison to 20 °C. Consequently, the shoot ratio based on total plant dry mass was reduced by 1.9% at 15 °C. Results of Exp-2 (Table 3) indicated that in general, shoot growth of plants including FW, DW and height, was distinctly affected by root cooling. Plant biomass increased as plants aged but the negative impact of low root temperature appeared seven days after initiation of treatment (Figure 2). The negative effect on shoot biomass was dependent on the treatment duration (Figure 2) and the final yield was 26% lower at 10 °C than 20 °C (Table 3). The root growth also lagged behind at 10 °C compared to the warmer treatment, and the mean FW and DW indicated that 10 °C treated plants contained less root at 43 DAS (Figure 2). However, the differences disappeared at the second harvest. In the last harvest, root biomass (FW and DW) was higher at 10 °C than the warmer treatment, but not statistically detected in both FW ( $p = 0.055$ ) and DW ( $p = 0.180$ ) (Table 3). Based on the increase in root DW and decrease in shoot DW, the shoot ratio of plants grown at 10 °C increased by 2% by the final harvest.

**Table 3.** Yield, shoot and root fresh weight (FW), shoot and root dry weight (DW), shoot height, root length and shoot ratio of Chinese broccoli under different root temperatures in Exp-1 and Exp-2. Significant differences ( $p < 0.05$ ) are indicated in bold.

		Exp-1			Exp-2		
	Treatment	15 °C	20 °C	<i>p</i> -Value	10 °C	20 °C	<i>p</i> -Value
Shoot	Yield (g)	153.5 ± 21.2	154.7 ± 22.2	0.878	137.2 ± 9.0	172.9 ± 32.2	<b>0.041</b>
	FW (g)	170.4 ± 21.6	175.5 ± 21.6	0.511	153.4 ± 13.7	218.0 ± 24.7	<b>&lt;0.001</b>
	DW (g)	14.83 ± 2.16	15.38 ± 2.37	0.499	14.52 ± 0.99	17.40 ± 2.09	<b>0.018</b>
	Height (cm)	35.30 ± 5.84	35.77 ± 4.53	0.802	34.35 ± 3.82	41.55 ± 4.35	<b>0.013</b>
	Shoot ratio (%)	92.94 ± 0.61	94.37 ± 0.86	<b>&lt;0.001</b>	93.47 ± 0.34	95.37 ± 1.22	<b>0.011</b>
Root	FW (g)	15.32 ± 2.37	11.28 ± 1.63	<b>&lt;0.001</b>	19.58 ± 1.23	16.67 ± 2.83	0.055
	DW (g)	1.12 ± 0.16	0.91 ± 0.15	<b>&lt;0.001</b>	1.01 ± 0.06	0.85 ± 0.26	0.180
	Length (cm)	29.43 ± 5.07	26.73 ± 5.73	0.177	31.30 ± 2.12	35.52 ± 3.35	<b>0.029</b>

Shoot ratio: shoot dry weight divided by the total plant dry weight, expressed in %.



**Figure 2.** Effects of root temperature on shoot and root fresh weight, shoot and root dry weight, shoot height and root length of Chinese broccoli at three harvest dates (43 DAS, 50 DAS and 57 DAS) during Exp-2. Levels of significance are represented by ns not significant, \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ . DAS: Days after sowing.

### 3.3. Effects of Root Temperature on Elemental Composition

In Exp-1, C and P concentrations on a dry weight basis of the whole plants were not sensitive to different root temperatures (Table 4). The concentrations of N, K and Mg in the root were higher at 15 °C than 20 °C, whereas Ca concentration was lower. Only Mg concentration of leaves and stems were enhanced at 15 °C and behaved in accordance with the root. In Exp-2, C concentration of plant fluctuated during plant growth, however, the pattern of changes differed according to different plant parts and root temperature treatment as shown in Table 4. In general, plants at 10 °C root temperature accumulated more carbon in stems and leaves at three harvest dates. The differences between 10 and 20 °C root temperature in the stem were highest (7.7 %) at 50 DAS and lowest (2.6%) at 57 DAS. Compared to the control group (20 °C), the increased carbon level percentage of leaves at 10 °C decreased gradually from 9.1% to 4.0% with plant aged. No differences were observed between the two root temperatures in the C level of the root. In contrast to the carbon level, N concentration was generally lower at 10 °C in stems and leaves for all time points, and the decrease became less significantly with crop age. However, the nitrogen level of the roots was higher at 10 °C throughout the experiment. Phosphorus concentrations were lower in the stems and leaves at 10 °C, but higher in the roots, compared to 20 °C. Differences in phosphorus between the two groups also showed rebound or some level of recovery as plants aged. Likewise, K, Ca and Mg levels of the leaves were lower at 10 °C. The differences started at 7 days after treatment and disappeared afterward. The results of K, Ca, Mg in the stems were different, with the highest differences at 50 DAS. The impact of low root temperature (10 °C) on K, Ca, Mg level in the roots was positive after seven days of root cooling and behaved differently in the later stages. Ca concentration in the roots was not sensitive to root temperatures throughout the experiment.

**Table 4.** 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 ( $p < 0.05$ ) are indicated in bold. DAS: days after sowing. DW: dry weight.

(% DW)		Exp-1						Exp-2					
		53 DAS			43 DAS			50 DAS			57 DAS		
		15 °C	20 °C	<i>p</i> -Value	10 °C	20 °C	<i>p</i> -Value	10 °C	20 °C	<i>p</i> -Value	10 °C	20 °C	<i>p</i> -Value
Leaves	C	38.75 ± 0.58	38.63 ± 0.63	0.583	39.89 ± 0.47	36.55 ± 0.62	<b>&lt;0.001</b>	37.91 ± 0.89	34.86 ± 0.56	<b>&lt;0.001</b>	38.28 ± 0.84	36.81 ± 0.99	<b>0.021</b>
	N	6.35 ± 0.15	6.39 ± 0.11	0.361	5.32 ± 0.09	6.34 ± 0.12	<b>&lt;0.001</b>	5.06 ± 0.21	6.19 ± 0.12	<b>&lt;0.001</b>	5.4 ± 0.41	5.93 ± 0.29	<b>0.028</b>
	P	0.63 ± 0.07	0.62 ± 0.04	0.887	0.48 ± 0.04	0.66 ± 0.06	<b>0.001</b>	0.52 ± 0.07	0.62 ± 0.05	<b>0.032</b>	0.69 ± 0.04	0.78 ± 0.06	<b>0.011</b>
	K	2.89 ± 0.29	2.85 ± 0.27	0.682	5.01 ± 0.45	6.14 ± 0.21	<b>0.003</b>	6.55 ± 1.15	6.18 ± 0.82	0.582	5.69 ± 0.84	5.31 ± 0.62	0.400
	Ca	3.62 ± 0.50	3.38 ± 0.42	0.159	4.07 ± 0.46	5.94 ± 0.50	<b>&lt;0.001</b>	4.41 ± 0.81	5.63 ± 0.74	<b>0.038</b>	4.35 ± 1.12	5.05 ± 0.95	0.270
	Mg	0.55 ± 0.06	0.46 ± 0.04	<b>&lt;0.001</b>	0.62 ± 0.07	0.91 ± 0.06	<b>&lt;0.001</b>	0.68 ± 0.12	0.79 ± 0.11	0.157	0.73 ± 0.15	0.74 ± 0.12	0.889
Stems	C	38.54 ± 0.97	38.09 ± 1.13	0.235	37.45 ± 0.63	35.57 ± 0.58	<b>0.001</b>	39.7 ± 0.95	36.86 ± 0.87	<b>0.001</b>	38.96 ± 0.69	37.2 ± 0.88	<b>0.004</b>
	N	4.53 ± 0.35	4.35 ± 0.18	0.094	2.94 ± 0.22	4.01 ± 0.18	<b>&lt;0.001</b>	3.88 ± 0.17	4.32 ± 0.23	<b>0.010</b>	3.69 ± 0.16	3.94 ± 0.14	<b>0.016</b>
	P	0.49 ± 0.04	0.51 ± 0.05	0.293	0.38 ± 0.06	0.53 ± 0.05	<b>0.003</b>	0.61 ± 0.03	0.65 ± 0.04	0.125	0.56 ± 0.02	0.57 ± 0.06	0.130
	K	5.52 ± 0.49	5.52 ± 0.59	0.992	7.45 ± 1.30	8.12 ± 0.77	0.360	7.73 ± 1.05	10.58 ± 1.08	<b>0.003</b>	7.4 ± 0.44	8.28 ± 1.23	0.146
	Ca	0.86 ± 0.17	0.84 ± 0.19	0.663	1.13 ± 0.26	1.12 ± 0.08	0.990	1.13 ± 0.12	1.69 ± 0.32	<b>0.014</b>	1.17 ± 0.17	1.48 ± 0.22	0.020
	Mg	0.29 ± 0.03	0.26 ± 0.03	<b>0.048</b>	0.39 ± 0.07	0.40 ± 0.04	0.773	0.45 ± 0.06	0.55 ± 0.04	<b>0.014</b>	0.44 ± 0.06	0.52 ± 0.09	0.122
Roots	C	37.30 ± 0.90	37.18 ± 1.86	0.826	44.9 ± 0.29	45.15 ± 0.35	0.267	45.38 ± 0.37	43.5 ± 2.32	0.146	45.90 ± 0.32	45.51 ± 0.41	0.098
	N	5.44 ± 0.22	4.97 ± 0.28	<b>&lt;0.001</b>	5.4 ± 0.44	4.46 ± 0.26	<b>0.006</b>	5.17 ± 0.15	3.82 ± 0.55	<b>0.004</b>	4.44 ± 0.37	3.82 ± 0.54	<b>0.045</b>
	P	0.95 ± 0.14	0.96 ± 0.11	0.69	0.69 ± 0.16	0.46 ± 0.07	<b>0.029</b>	0.45 ± 0.05	0.38 ± 0.11	0.220	0.45 ± 0.04	0.36 ± 0.05	<b>0.009</b>
	K	0.43 ± 0.05	0.37 ± 0.04	<b>&lt;0.001</b>	0.30 ± 0.07	0.19 ± 0.03	<b>0.015</b>	0.26 ± 0.15	0.34 ± 0.11	0.384	0.30 ± 0.14	0.25 ± 0.13	0.454
	Ca	0.63 ± 0.08	0.73 ± 0.11	<b>0.003</b>	0.78 ± 0.09	0.81 ± 0.07	0.636	0.64 ± 0.07	0.64 ± 0.11	0.957	0.69 ± 0.09	0.72 ± 0.13	0.624
	Mg	0.38 ± 0.05	0.26 ± 0.03	<b>&lt;0.001</b>	0.35 ± 0.05	0.22 ± 0.02	<b>0.002</b>	0.26 ± 0.05	0.15 ± 0.02	<b>0.008</b>	0.27 ± 0.03	0.18 ± 0.03	<b>&lt;0.001</b>



### 3.4. Effects of Root Temperature on Soluble Sugars, Total Chlorophyll and Starch

The accumulated amounts of organic compounds with the results of statistical analyses are listed in Table 5. In Exp-1, the root temperature treatments showed no differences in soluble sugars and starch, except for the significant increase of sucrose in the leaves at 15 °C root temperature. Total chlorophyll concentration in the stems ( $p < 0.001$ ) and leaves ( $p = 0.038$ ) strongly increased at reduced root temperatures. Results of Exp-2 indicated that glucose and fructose concentration of the stems and leaves increased in older plants. Except for stems at a 10 °C root temperature, glucose levels indicated a reduction potential for the first 7 days, followed by an increase in the second 7 days. Relative to the control root temperature (20 °C), a lower root temperature (10 °C) enhanced the accumulation of soluble sugars and total chlorophyll in leaves and stems. Glucose and fructose concentrations were most affected by root temperature at 43 DAS and 50 DAS in leaves and at 43 DAS in stems, respectively (Table 5). The reaction of sucrose to low root temperature gradually reduced as the plants aged, and no differences were detected in the final harvest. The total chlorophyll concentration of stems increased as the plants aged, but no statistical differences were revealed between the two treatments. An increase of 23.5% and 31.8% in leaf chlorophyll concentration was observed after 7 and 14 days, respectively, of low root temperature application compared to the control group, but the differences disappeared in the last harvest. Due to the small sample size, samples from the first harvest were not sufficient for starch measurement. Based on the results of the last two harvests, only starch of the stems increased significantly in response to a low root temperature.

### 3.5. Effects of Root Temperature on Glucosinolates

Six aliphatic glucosinolates were detected in both stems and leaves of Chinese broccoli in two experiments (Table 6). Leaves contained higher levels of total glucosinolates than stems across all the treatments in both experiments. The most abundant glucosinolate was gluconapin, followed by sinigrin and glucobrassicin. Some glucosinolates, such as glucoerucin, were detected in only a few plants and in trace amounts (data not shown).

As shown in Table 6, the results of Exp-1 indicated the concentration of total glucosinolates, total aliphatic glucosinolates and total indolic glucosinolates in stems and leaves were not affected by the root temperatures tested. However, neoglucobrassicin and glucoiberin of leaves and glucoiberin of stems were significantly greater in the Chinese broccoli at a 15 °C root temperature than the warmer temperature. In Exp-2, after 14 days of treatment (50 DAS), root temperature significantly affected the concentration of total glucosinolates, total aliphatic, total indolic and individual glucosinolates. Under a lower root temperature of 10 °C, the total glucosinolate concentration in the leaves increased 150.5%. For individual glucosinolates, such as glucobrassicin, neoglucobrassicin, progoitrin, glucoraphanin and gluconapin, the levels were almost triple at a 10 °C than 20 °C root temperature. However, after 21 days (57 DAS), the increase was only observed in the concentration of 4-hydroxyglucobrassicin, neoglucobrassicin and sinigrin with 82.6%, 93.5% and 72.6%, respectively. Stems tended to be less sensitive to root temperature compared to the leaves. The increase in the stems was only detected in the individual glucosinolates: 4-hydroxyglucobrassicin (55.1 %) and progoitrin (58.8%) at 50 DAS, and in the final harvest, all the glucosinolate concentrations were similar between the two root temperatures.

**Table 5.** 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 ( $p < 0.05$ ) are indicated in bold. DAS: days after sowing. FW: fresh weight.

(mg/g FW)		Exp-1						Exp-2					
		53 DAS			43 DAS			50 DAS			57 DAS		
		15 °C	20 °C	<i>p</i> -Value	10 °C	20 °C	<i>p</i> -Value	10 °C	20 °C	<i>p</i> -Value	10 °C	20 °C	<i>p</i> -Value
Leaves	Glucose	1.18 ± 0.35	1.44 ± 0.45	0.083	1.87 ± 0.52	0.52 ± 0.12	<b>0.004</b>	2.83 ± 1.22	0.82 ± 0.08	<b>0.021</b>	3.50 ± 1.43	1.41 ± 0.46	<b>0.014</b>
	Fructose	1.78 ± 0.65	2.10 ± 0.47	0.133	1.00 ± 0.52	0.27 ± 0.01	<b>0.034</b>	2.25 ± 1.20	0.48 ± 0.21	<b>0.028</b>	3.51 ± 1.18	1.43 ± 0.34	<b>0.006</b>
	Sucrose	1.38 ± 0.19	0.99 ± 0.20	<b>&lt;0.001</b>	2.20 ± 0.58	0.58 ± 0.17	<b>0.002</b>	0.71 ± 0.14	0.62 ± 0.30	0.570	0.91 ± 0.38	1.00 ± 0.21	0.626
	Chlorophyll	4.74 ± 1.10	3.97 ± 0.80	<b>0.038</b>	2.84 ± 0.13	2.30 ± 0.10	<b>&lt;0.001</b>	5.84 ± 0.30	4.43 ± 0.57	<b>0.003</b>	5.37 ± 0.97	5.41 ± 0.76	0.937
	Starch	2.55 ± 1.22	2.15 ± 1.07	0.341				2.10 ± 0.65	0.95 ± 0.56	<b>0.017</b>	1.31 ± 0.49	0.55 ± 0.24	<b>0.011</b>
Stems	Glucose	7.81 ± 0.99	7.64 ± 1.00	0.649	8.27 ± 1.41	3.78 ± 0.53	<b>0.001</b>	7.43 ± 0.90	5.83 ± 1.25	0.052	9.37 ± 1.46	7.21 ± 0.77	<b>0.013</b>
	Fructose	6.57 ± 0.82	6.49 ± 0.80	0.788	3.40 ± 1.45	1.04 ± 0.27	<b>0.020</b>	6.86 ± 0.72	4.62 ± 0.77	<b>0.001</b>	8.06 ± 0.88	6.29 ± 0.76	<b>0.004</b>
	Sucrose	1.91 ± 0.56	1.98 ± 0.53	0.697	7.50 ± 1.18	4.14 ± 0.80	<b>0.001</b>	3.77 ± 1.44	2.60 ± 0.59	0.150	3.41 ± 1.58	3.29 ± 1.32	0.886
	Chlorophyll	0.48 ± 0.19	0.19 ± 0.06	<b>&lt;0.001</b>	0.31 ± 0.11	0.23 ± 0.08	0.237	0.33 ± 0.10	0.28 ± 0.05	0.321	0.38 ± 0.09	0.29 ± 0.07	0.080
	Starch	0.23 ± 0.09	0.24 ± 0.05	0.607				0.35 ± 0.23	0.37 ± 0.21	0.915	0.29 ± 0.08	0.21 ± 0.05	0.069

**Table 6.** Total, aliphatic, indolic and individual glucosinolates concentrations ( $\mu\text{mol}/100\text{g}$  FW) in the leaves and stems of Chinese broccoli under different root temperatures and harvest dates in Exp-1 and Exp-2. Significant differences ( $p < 0.05$ ) are indicated in bold. DAS: days after sowing. FW: fresh weight.

(μmol/100g FW)		Exp-1			Exp-2					
		53 DAS			50 DAS			57 DAS		
		15 °C	20 °C	<i>p</i> -Value	10 °C	20 °C	<i>p</i> -Value	10 °C	20 °C	<i>p</i> -Value
Leaves	Sinigrin	14.66 ± 7.02	12.71 ± 7.15	0.506	15.35 ± 3.22	6.37 ± 1.91	<b>0.002</b>	18.11 ± 4.98	10.49 ± 3.65	<b>0.014</b>
	Progoitrin	1.39 ± 0.55	1.88 ± 1.54	0.322	1.46 ± 0.18	0.57 ± 0.06	<b>&lt;0.001</b>	1.74 ± 0.69	0.98 ± 0.52	0.059
	Glucoraphanin	2.21 ± 1.76	1.35 ± 0.94	0.152	1.18 ± 0.31	0.30 ± 0.15	<b>0.001</b>	3.93 ± 1.80	2.92 ± 1.54	0.322
	Gluconapin	31.75 ± 16.74	34.76 ± 25.24	0.735	46.67 ± 10.77	19.07 ± 5.95	<b>0.002</b>	57.05 ± 20.01	41.17 ± 18.87	0.188
	Glucoiberin	1.77 ± 0.86	0.82 ± 0.56	<b>0.005</b>	0.43 ± 0.12	0.18 ± 0.08	<b>0.009</b>	1.49 ± 0.62	1.32 ± 0.54	0.626
	Glucoalyssin	0.05 ± 0.04	0.05 ± 0.03	0.710	0.10 ± 0.02	0.06 ± 0.01	<b>0.012</b>	0.19 ± 0.07	0.13 ± 0.07	0.135
	Total	51.83 ± 26.60	51.57 ± 33.14	0.983	65.61 ± 14.51	26.72 ± 8.03	<b>0.002</b>	84.00 ± 26.66	58.33 ± 24.98	0.116
	Glucobrassicin	5.12 ± 2.80	4.66 ± 2.51	0.678	16.36 ± 7.97	5.79 ± 1.62	<b>0.040</b>	10.41 ± 3.33	6.96 ± 2.40	0.069
	4-Methoxyglucobrassicin	0.53 ± 0.11	0.50 ± 0.14	0.557	0.52 ± 0.14	0.34 ± 0.03	<b>0.036</b>	0.58 ± 0.15	0.49 ± 0.07	0.201
	Neoglucobrassicin	0.21 ± 0.14	0.09 ± 0.09	<b>0.024</b>	0.79 ± 0.31	0.27 ± 0.12	<b>0.017</b>	0.42 ± 0.18	0.23 ± 0.05	<b>0.044</b>
	4-Hydroxyglucobrassicin	1.15 ± 0.26	1.08 ± 0.27	0.502	0.97 ± 0.27	0.50 ± 0.07	<b>0.017</b>	1.49 ± 0.44	0.77 ± 0.23	<b>0.008</b>
	Total	7.01 ± 3.07	6.33 ± 2.69	0.570	18.64 ± 8.50	6.90 ± 1.70	<b>0.035</b>	12.90 ± 3.98	8.44 ± 2.65	<b>0.049</b>
	Total	58.84 ± 29.33	57.89 ± 35.68	0.944	84.25 ± 22.02	33.63 ± 9.44	<b>0.004</b>	96.90 ± 30.10	66.78 ± 27.55	0.101
Stems	Sinigrin	14.28 ± 3.29	12.17 ± 4.98	0.236	27.00 ± 4.82	22.14 ± 6.28	0.210	14.81 ± 6.67	15.41 ± 4.26	0.856
	Progoitrin	2.88 ± 0.99	2.68 ± 0.78	0.591	5.70 ± 1.21	3.59 ± 1.02	<b>0.018</b>	2.92 ± 1.66	2.55 ± 0.87	0.645
	Glucoraphanin	6.35 ± 2.17	5.37 ± 1.24	0.191	6.97 ± 1.51	6.15 ± 2.42	0.543	6.46 ± 2.31	8.56 ± 2.73	0.181
	Gluconapin	33.75 ± 10.92	29.56 ± 7.61	0.289	71.72 ± 15.94	56.72 ± 22.02	0.256	31.96 ± 18.86	38.34 ± 13.58	0.518
	Glucoiberin	2.55 ± 1.00	1.64 ± 0.84	<b>0.025</b>	2.30 ± 0.71	2.06 ± 0.84	0.640	1.80 ± 1.11	2.28 ± 0.67	0.388
	Glucoalyssin	0.07 ± 0.03	0.07 ± 0.02	0.618	0.24 ± 0.04	0.23 ± 0.12	0.875	0.21 ± 0.06	0.23 ± 0.07	0.633
	Total	59.89 ± 17.14	51.49 ± 12.08	0.181	113.92 ± 23.31	90.89 ± 31.26	0.232	58.16 ± 30.26	67.38 ± 20.93	0.546
	Glucobrassicin	2.87 ± 1.12	2.33 ± 1.08	0.238	9.27 ± 2.97	8.62 ± 4.14	0.782	2.83 ± 1.81	4.00 ± 1.60	0.263
	4-Methoxyglucobrassicin	1.41 ± 0.21	1.51 ± 0.24	0.341	2.45 ± 0.47	2.31 ± 0.74	0.727	1.99 ± 0.37	2.34 ± 0.27	0.100
	Neoglucobrassicin	0.54 ± 0.26	0.36 ± 0.14	0.051	4.21 ± 1.54	2.59 ± 1.26	0.107	1.55 ± 1.04	1.47 ± 0.55	0.872
	4-Hydroxyglucobrassicin	2.51 ± 0.73	2.37 ± 0.43	0.577	1.94 ± 0.38	1.27 ± 0.42	<b>0.031</b>	2.45 ± 0.87	2.58 ± 0.39	0.753
	Total	9.57 ± 2.50	8.89 ± 2.54	0.514	17.87 ± 5.00	14.79 ± 6.02	0.406	8.83 ± 3.93	10.39 ± 2.41	0.429
	Total	60.46 ± 17.21	60.37 ± 13.24	0.162	131.78 ± 27.87	105.68 ± 36.95	0.247	66.99 ± 34.01	77.77 ± 23.15	0.533

#### 4. Discussion

Chinese broccoli is regarded as a cold tolerant and heat-sensitive plant, since it prefers to grow in cooler temperatures (15–25 °C), has short tolerance to low temperature (−2 °C) or frost without injury and is sensitive to high air temperature [30]. The specific optimum ambient air temperature depends on different growth stages: 25–30 °C for germination and adaption after transplanting; 15–20 °C for the development of leaves and stem bolting [30]. Compared to Exp-1, lower than optimal air temperatures after transplanting in Exp-2 could explain our observations of low growth rates and longer developmental periods. Yang and Yang [31] reported that low air temperatures (15–20 °C) promoted bolting, flower bud differentiation, and eventually improved the yield and quality of mature Chinese broccoli plants. Although “Cuimei F1” is a new variety, which can bolt without the stimulation of low air temperature, the increased yield (55%) observed at 20 °C root temperature in Exp-2 compared to Exp-1 could have been due to the positive effect of a lower air temperature during bolting.

Previous studies have indicated the negative impacts of suboptimal root temperature on root growth on carrots [9]. Possible mechanisms include alteration of turgor pressure, phytohormone production and altered cell wall properties [32]. The increased root biomass in Exp-1 may be explained by lower metabolic and respiration rates at low root temperature [33], which reduced carbohydrates consumption in the root and promoted higher root development. Since Chinese broccoli are cool season plants, another explanation could be that a 15 °C root temperature favors growth more than a warmer temperature. Zhang et al. [34] also showed that cold-tolerant plants such as figleaf gourd and turban squash grew better at 14 °C than at higher root temperatures. In Exp-2, root biomass was reduced after exposure of the roots to 10 °C for seven days with no difference in the following 14 days (Table 3). We were unable to find published reports about the optimal root temperature for Chinese broccoli. The reduction of root dry biomass proved that 10 °C in the present Exp-2 was in the suboptimal range of Chinese broccoli. A low root temperature may have reduced the sink strength of root growth, decreasing the translocation of photoassimilates to the roots, which lead to lower root growth.

Restriction of shoot growth by suboptimal root temperatures is well-known [35]. Yan et al. [7] showed that cucumber seedlings with roots immersed in a 12 °C nutrition solution had less total plant dry weight than seedlings with roots at 20 °C. At 15 °C root temperature, *Valerianella locusta* plants had smaller shoots than plants at 20 °C [36]. Exposure of red leaf lettuce to low root temperature (10 °C) significantly reduced the fresh weights of the shoots [8]. The effects of low root temperature on shoot growth have been attributed to decreased water uptake resulting from a number of factors including: lower hydraulic conductance caused by lower vapor pressure difference between leaves and air; cell wall alteration caused by lipid peroxidation and decreased induction of suberin layers; and reduced photosynthesis caused by the closure of the stomata [37,38]. Root phytohormone production including ABA could be altered by temperature, influencing shoot to root hormone signaling and shoot production [39]. Paul and Foyer [40] mentioned that reduced sink strength of the roots at lower root temperature caused the accumulation of photosynthesis assimilates, which in turn down-regulated genes involved in photosynthesis. In accordance with the aforementioned studies, we found that the fresh weight of shoots was significantly reduced by low root temperature (10 °C) in Exp-2. The lower shoot ratio of both experiments indicated that root cooling channeled more photoassimilates to the growth of the root. Equiza [41] interpreted the reduction in the shoot ratio as a mechanism to overcome restrictions in water absorption caused by low root temperature. In contrast, He et al. [33] observed a higher shoot ratio in lettuce at a cool root temperature (20 °C) in hot tropical regions. Therefore, the effect of root temperature on biomass is dependent on the surrounding air temperatures and other climatic factors.

Cationic minerals of vegetables provide essential elements in dietary sources. It was generally accepted that both uptake and transport of macro- and micro-nutrients are reduced in response to low root temperatures [42]. Here we showed in Exp-1, that N, K and Mg concentrations in the root increased in response to root cooling. In addition, the increased P was also observed in Exp-2 after 7 days of treatment. These increases are in line with Pettersson [43], who found that the K net uptake

rate of barley at 10 °C root temperature was 15%–25% higher than at 25 °C, and K, N and Mg tended to accumulate in the root. In agreement with our findings, this accumulation in roots of plants could be described as nutrient storage for future use. Adebooye et al. [5] found that at sup-optimal root temperature, the roots of African snake tomato contained higher amounts of Ca. In contrast with Adebooye et al. [5], our results show that Ca in the roots was either reduced or remained stable at lower root temperatures. An explanation might be that the redistribution efficiency of Ca is generally low within the plant, even under root temperature stress. In addition, competition with K and Mg may lead to a lower concentration of Ca in the root and shoot [44]. Essential elements such as P, N, K, Mg, Ca were reduced to a varying degree in Chinese broccoli leaves and stems when their roots were exposed to 10 °C in Exp-2 (Table 4). Mineral concentrations in the shoot are dependent on two processes: the uptake by roots and translocation from roots to shoots [45]. Our results indicated that the reduction of elements in the shoot might be due to the lower translocation rate since most of them accumulated in the root.

Soluble sugar levels affect the taste and consumer acceptance of Chinese broccoli [11]. Our results of carbohydrate concentration are consistent with the work of Rosa et al. [11], which showed that fructose and glucose are the major soluble sugars, and starch exists only in a minor amount in Brassica vegetables. Sugar accumulation at cold root temperatures has been widely documented in many plants including lettuce [33], red leaf lettuce [8], spinach [46], and cocktail tomato [26]. An increase of sucrose in the leaves of Chinese broccoli in Exp-1 as well as glucose and fructose accumulation in leaves and stems in Exp-2 are in accordance with previous findings. Increased carbohydrates in the shoot can be explained by the reduced sink strength of the root in response to low root temperature. In addition, accumulated soluble sugars and starch in the shoot can be considered as a defensive mechanism of the plant to exposure to cold stress, since sugars can be used as osmoprotectants, as well as energy molecules and primary messengers of stress [47].

Chlorophyll is an important pigment and its concentration affects the visual quality of Chinese broccoli. Low air temperature is in general believed to suppress the biosynthesis of photosynthetic pigments due to photo-oxidation damage [5,48]. Chloroplasts are the main cold sensors of plants and produce most reactive oxygen species (ROS), including hydroxyl radicals, superoxide, hydrogen peroxide, in response to low air temperature [49]. ROS can cause chloroplast damage and therefore reduce chlorophyll levels [50]. However, Gazula et al. [51] reported that chlorophyll increased in three lettuce cultivars at lower air temperatures. Also, Kalisz et al. [52] observed that the level of chlorophyll a and b in basil remained stable or even increased in one red basil cultivar at 6 °C compared to 18 °C, although net photosynthetic rates were significantly reduced. They attributed the accumulation of chlorophyll to acclimation mechanisms during the treatment. The root environment could thus influence photosynthesis by affecting stomatal conductance or by metabolic impairment [34], but similar to air temperature, the effects of root temperature on chlorophyll levels are sometimes contradictory. Enhanced chlorophyll concentrations were observed of African snake tomato in response to elevated root temperature [5,51]. High root temperature stress reduced the amount of chlorophyll concentration in the leaves of carrot [9]. In the experiments of Sun et al. [2] with lettuce (*Lactuca sativa* L.) and Nguyen et al. [53] with coriander, total chlorophyll content was not affected by root cooling. In the present Exp-2, a reduction in root temperature from 20 °C to 10 °C caused a marked increase in the total chlorophyll concentration of the leaves, the highest increase being after 7 days of treatment. This increase could be due to the cold acclimation of plants, as Kalisz et al. [52] suggested for basil. Root cooling to 15 °C significantly increased the total chlorophyll level in stems and leaves in Exp-1, suggesting improvement to photosynthesis and light harvesting apparatus [2]. However, further research on the reaction of photosynthesis and transpiration to root temperature needs to be conducted.

Glucosinolates are important health-promoting components in Chinese broccoli. In this study, ten individual glucosinolates were detected in stems and leaves of Chinese broccoli, of which the predominant was gluconapin, followed by sinigrin and glucobrassicin. In agreement with previous studies, aliphatic glucosinolates were much more abundant than indolic glucosinolates in stems and

leaves [15,54,55]. However, no aromatic glucosinolates were detected in both trials. In contrast to our studies, previous studies showed that young leaves of Chinese broccoli had less total glucosinolates than bolting stems [25]. This may be due to different cultivars or cultivation regimes. Glucosinolate levels have been reported to be largely affected by surrounding environmental factors, such as salinity, drought, light, or air temperature [56]. The effects of air temperature on glucosinolate concentration in other Brassicaceae species have been reported, but with varying results. It is frequently stated that the use of suboptimal air temperatures for growth-induced, higher levels of glucosinolates is based on antioxidant effects against ROS [57,58]. Engelen-Eigles et al. [59] reported that watercress (*Nasturtium officinale*) accumulated more gluconasturtiin when grown at 10 or 15 °C than at 20 °C or 25 °C. *Arabidopsis thaliana* contained higher levels of total glucosinolates at 9 or 15 °C air temperatures than at warmer temperatures, and aliphatic glucosinolates were subject to air temperature [60]. Similarly, in *Brassica oleracea* L., higher glucosinolate levels in the leaves were obtained when plants were moved from 32 to 12 °C compared to being kept at a constant 32 °C [61]. On the other hand, Guo et al. [62] reported higher glucoraphanin in broccoli sprouts grown at 25 °C than at 20 or 30 °C. Another study on cabbage seedlings (*Brassica oleracea* var. *capitata*) by Rosa and Rodrigues [63] found no correlation between air temperature and glucosinolate concentration. It was therefore assumed that the effects of air temperature were associated with plant organs, species and temperature being tested [19]. There are not many studies about the impacts of root temperature on glucosinolate levels. Wild rocket (*Diplotaxis tenuifolia* cv. Frastagliata) had reduced levels of aliphatic glucosinolates, but no effects on aromatic and indolic glucosinolates, after 48 h of root heating at 40 °C [64]. This may have been due to breakdown of glucosinolates upon tissue damage caused by heating [65]. In the present study, a root temperature as low as 15 °C, increased the individual and total glucosinolate concentration. These results further proved that plants grown at suboptimal root temperatures or under cold root temperature stress tend to accumulate more glucosinolates. Our results showed indolic glucosinolates responded strongly to root cooling, especially neoglucobrassin and 4-hydroxyglucobrassicin. This is in agreement to a study on broccoli where indolic glucosinolates were more sensitive to environmental factors [66]. However, previous studies on *Arabidopsis thaliana* [60] and kale [19], concluded that aliphatic glucosinolates were more affected by growth temperatures. Our results suggest that low root temperatures can increase the concentration of glucosinolates. However, similar to air temperature, the effect of root temperature depends highly on other factors, such as plant organ, plant development and plant species.

The results in Exp-2 indicated the kinetics of cold acclimation during the 21 days of 10 °C root temperatures. Barrero-Gil et al. [67] did not find an increase in sugars after 2 days of 10 °C cold treatment and suggested that the accumulation of sugars was important for chilling acclimation. Aydin et al. [57] observed that short-term responses of tomato to cold maximised antioxidant enzyme, which was present after 22 days. Ntatsi et al. [68] and Yang et al. [69] described this long-term process as adaptive and time-dependent, with the accompanying down-regulation of secondary metabolism in tomato roots after long-term cold treatment. In the review by Ruelland et al. [70], photoinhibition is only observed in the short-term, due to enzymatic reactions of low temperature. With extension of cooling time, photosynthesis recovers, new leaves grow and metabolism adapts to the new thermal conditions. The gradual decline in differences between the two groups in Exp-2 here also supports conclusions of previous studies.

## 5. Conclusions

In the present work, we investigated if reducing root temperature affects product quality of Chinese broccoli. The results showed that a lower root temperature at 15 °C slightly enhanced the concentrations of sucrose, chlorophyll and two individual glucosinolates (glucoiberin and neoglucobrassicin) in the bolting stems without influencing the yield. In contrast, at 10 °C, the levels of soluble sugars and glucosinolates in the shoot were strongly enhanced, but the yield was reduced at the same time. Because these parameters correlate with the sensory descriptors of good flavor and healthy parameters, a lower root temperature most likely has a positive effect on Chinese broccoli taste and antioxidants



levels. Nutritional properties are of less interest to farmers than yield. However, nutritional values are important factors to customers because of perceived health benefits. A feasible method to improve the food quality of Chinese broccoli could be manipulation of root temperature. Further studies should focus on how to improve quality but minimize yield loss.

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