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Effects of root temperature on food quality of horticultural crops

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Abstract

Different from ambient temperature, root temperature is convenient and economical to manage and control, especially with the development of greenhouse. Understanding the effects of root temperature on plant growth and key food components of horticultural crops under greenhouse conditions is important because of its high economic interest. In Chapter 2, 3 and 4, growth and food quality (sugar, antioxidants and minerals) of cocktail tomato (*Lycopersicon esculentum* cv “Amoroso” and cv “Delioso”) and Chinese broccoli (*Brassica oleracea* var. *alboglabra* cv “Cuimei”) under different root temperature treatments were investigated.

For cocktail tomatoes, reductions of marketable yield per plant in both cultivars were observed in response to root cooling (10°C) in winter, but not significantly in summer, compared to control group (16-27°C). In most cases, root cooling had a positive effect on the sensory and nutritional quality (sugars, vitamin C and carotenoids levels) of cocktail tomatoes. Specifically, 'Delioso' showed an increase in glucose, vitamin C and lycopene concentration of the fruits after root cooling in both seasons, while 'Amoroso' exhibited only higher consistent values in glucose levels. For Chinese broccoli, low root temperature (10 and 15°C) was in general associated with a higher concentration of soluble sugars, total chlorophyll and glucosinolates, but lower mineral levels in stems and leaves than the control group (20°C), regardless of the treatment duration. The yield was reduced with root cooling, but shortening the cooling treatment alleviated this reduction, especially in summer.

Manipulation of root temperature could be a feasible method to improve the overall food quality of cocktail tomatoes and Chinese broccoli. However, this effect is dependent on cultivars and other environmental factors.

Zusammenfassung

Anders als die Umgebungstemperatur bei der Pflanzenproduktion lässt sich die Wurzeltemperatur bequem und wirtschaftlich steuern und kontrollieren, insbesondere unter Gewächsausbedingungen. Dabei ist das Verständnis der Auswirkungen der Wurzeltemperatur auf das Pflanzenwachstum und auf den Gehalt wichtiger Inhaltsstoffe in Gartenbaukulturen von grundlegender Bedeutung und kann von hohem wirtschaftlichem Interesse sein. In Kapitel 2, 3 und 4 wurden das Wachstum und die Qualität (z.B. Gehalte an Zucker, Antioxidantien und Mineralien) von Cocktailtomaten (*Lycopersicon esculentum* cv "Amoroso" und cv "Delioso") und Chinesischem Brokkoli (*Brassica oleracea* var. *Alboglabra* cv "Cuimei") unter verschiedenen Wurzeltemperaturbehandlungen im Gewächshaus untersucht.

Bei den Cocktailtomaten wurde bei beiden Sorten als Reaktion auf eine Wurzelkühlung (10°C) im Winter eine Verringerung des marktfähigen Ertrags pro Pflanze beobachtet, dies war im Sommer nicht signifikant nachweisbar, im Vergleich zur Kontrollgruppe (16-27°C). In den meisten Fällen wirkte sich die Wurzelkühlung positiv auf die sensorische und ernährungsphysiologische Qualität (Zucker, Vitamin C und Carotinoide) von Cocktailtomaten aus. Insbesondere zeigte "Delioso" einen Anstieg der Glukose-, Vitamin C- und Lycopin-Konzentration der Früchte nach Wurzelkühlung in beiden Jahreszeiten, während "Amoroso" nur höhere konsistente Werte bei den Glukosespiegeln aufwies. Beim Chinesischen Brokkoli war eine niedrige Wurzeltemperatur (10 und 15°C) im Allgemeinen mit einer höheren Konzentration an löslichen Zuckern, Gesamtchlorophyll und Glucosinolaten verbunden, jedoch mit niedrigeren Mineralstoffgehalten in Stielen und Blättern im Vergleich zur Kontrollgruppe (20°C), unabhängig von der Behandlungsdauer. Die Ausbeute wurde bei Wurzelkühlung verringert, eine Verkürzung der Kühlbehandlung auf eine Woche reduzierte diesen Effekt, insbesondere im Sommer.

Die Manipulation der Wurzeltemperatur erwies sich somit als eine praktikable Methode um die Gesamtnahrungsmittelqualität von Cocktailtomaten und Chinesischem Brokkoli zu verbessern. Die Größe des Effektes hängt dabei von den gewählten Pflanzensorten und von weiteren Umweltfaktoren ab.

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Chapter 1

General introduction

1.1 Root temperature and its influences

As an important factor of the plant's surrounding environment, root temperature or soil temperature has been studied since the last century. Roots are more sensitive to temperature fluctuations compared to other parts of the plant (Ahn et al., 1999). Root temperature not only impacts plant growth and biomass allocation but also causes changes in plant morphology and physiology (Füllner et al., 2012). In the review of Cooper (1973), he summarized the influence of root temperature on plant growth and processes, such as biomass, photosynthesis and water absorption, and concluded that almost all the species were characterized by a base, optimal and maximum root temperature.

In nature, soil temperature is related to but also varies from air temperature (McMichael and Burke 1998). Root temperature in the field is spatially and temporally heterogeneous, which is caused by differences in heat conduction and convection with rooting depth and at the soil surface (Füllner et al., 2012). This heterogeneity also influences other soil parameters, for example, nutrient availability, ion exchange capacity, oxygen and carbon dioxide distribution, number and species of soil microbes, which subsequently influences plant growth (McMichael and Burke 1998).

In the greenhouse, root temperature is mostly uniform spatially and temporally (Füllner et al., 2012). However, with the development of greenhouse techniques, including hydroponic systems, root temperature is regarded as an economical and convenient approach to regulate the growth and development of plants. Compared to the vast energy consumption,

root temperature management has been recommended to control plant growth. Recent research about manipulation of root temperature in the greenhouse could be summarized into two large categories: (1) Alleviation of the negative effects from sub- or supra-air temperature from greenhouse by heating or cooling the roots of the plants; (2) Enhancing the generation of valuable secondary metabolites in plants, especially fruits, vegetables or medical plants to achieve the best quality.

1.1.1 Plant growth and biomass

Plant growth inhibition was observed in many plants subjected to sub- or supra-optimal root temperature. Plant biomass, both root and shoot biomass of most species decreased when grown at suboptimal temperatures as noted in potato (Baghour et al., 2003), *Lotus japonicus* (Quadir et al., 2011), and *Betula pendula* (Solfjeld & Johnsen, 2006). In contrast, increasing root temperature to the optimum temperature could accelerate plant growth and improve elongation of individual roots and root branching (Beauchamp & Lathwell, 1967; McMichael & Quisenberry, 1993).

Considering the importance of root temperature on growth and biomass, manipulation of root temperature by root heating or cooling in the greenhouse could achieve better plants, especially at sub- or supra-optimal air temperature. For example, total plant dry weight increased when the roots of winter-grown green pepper were heated to 20 and 25°C (Ameen et al., 2019). Sweet pepper (*Capsicum annuum*) and poinsettia (*Euphorbia pulcherrima*) have been reported to show stem elongation with increased root temperature (Abdel-Mawgoud et al., 2005; Olberg & Lopez, 2016). Again, petunia growth was increased at lower air temperature (Olberg & Lopez, 2017). For tropical greenhouses, a root cooling system has been developed to alleviate air temperature stress (reviewed by Niam & Suhardiyanto, 2018). However, the effects of root cooling or heating on plant growth and biomass depend on

different species, the ambient air temperature, duration of treatment, and exact root temperature applied.

1.1.2 Plant physiology

Effects of root temperature on plant growth and development are attributed to the alteration of various essential physiological processes, such as photosynthesis, transpiration, nutrient uptake, water potential, stomatal conductance and root respiration. A large variety of results and mechanism on these aspects has been reported in the literature due to the diversity of experimental setups and species examined. Here, only some remarks are listed:

- Root morphology and respiration. The impacts of temperature on root structural modifications include alterations in membrane, cell wall hardening, reduction of root surface area, total root length and number of root tips (Aidoo et al., 2016). Reactive oxygen species (ROS) caused by lower root temperature stress results in the oxidation of root cellular components (Lee et al., 2004a & b). Zhang et al. (2007) reported evidence of peroxidation of root membrane lipids at lower root temperature in Cucurbit species. In addition, root temperatures affect the overall enzymatic activity of root systems and respiration (McMichael & Burke, 1998; Huang et al., 2005). At lower root temperature, the root respiration decreases with a Q_{10} (the proportional increase in respiration for every 10 °C rise in temperature) of approximately 2.0 over a limited temperature range (Atkin et al., 2000), and roots accumulated reducing sugar and nitrogen while consuming starch (Lunackova et al., 2000). Conversely, as temperature increases, root respiration increases and is generally modelled as increasing exponentially with temperature (Huang et al., 2005).
- Photosynthesis and transpiration. Root environment could easily influence photosynthesis by affecting stomatal conductance or metabolic impairment (Zhang et

al., 2008). The inhibition of growth at lower root temperature was often associated with a decrease in photosynthetic capacity (Malcolm et al., 2008). In tomato seedlings, net photosynthetic rate, transpiration rate and stomatal conductance were noticeably reduced at lower root temperature (He et al., 2014). In addition, net photosynthesis rate and photosystem IIphytochemicals were reduced when the roots of cucumber were exposed to temperatures below 15°C and even at optimal air temperature (Ahn et al., 1999). Again, photosynthetic rates of rice seedlings (Suzuki et al., 2008), cucumber seedlings (Anwar et al., 2019), and pepper (Aidoo et al., 2017) decreased in response to low root zone temperature. Possible reasons for the decreased photosynthesis under stress include a damaged apparatus (photo-damage of PSII), inhibition of CO_2 entry to mesophyll due to stomata closure, impaired biosynthesis of chlorophyll, suppression of CO_2 assimilation (Rubisco activity and RuBP regeneration) and metabolic constraints at low root temperature (Aidoo et al., 2016; He et al., 2013; Sun et al., 2016), which subsequently affects biomass production. He et al. (2013) also attributed the limitation of photosynthesis at low root temperature to the dysfunction of Rubisco protein given that Rubisco protein comprises 27% of total leaf N. More nitrogen was localized in the roots at suboptimal root temperature and further aggravated lower nitrogen concentration in the leaf (Lloyd et al., 2011). Since photosynthesis is sensitive to the equilibrium between C export and import, Ferrari et al. (2016) attributed the lower photosynthesis rate to the lower C fixation rate because the sink strength of root is lower in response to lower root temperature.

- Nutrient uptake. Ion absorption is an active and selective process that involves ion-specific transport proteins (pumps, transporters and channels) on root cell membranes, and ATP energy is produced through root respiratory process (Sago et al., 2011b).

Root temperature influences nutrient uptake based on its effect on one component of the process. Baghour et al. (2003) summarized the optimal soil temperature for most plants for uptake of most essential elements was 23-27°C, and Adebooye et al. (2010) believed root temperatures less than 20°C was sub-optimal. In the short term, low root temperature can affect nutrient uptake by changing root permeability or altering the structure and function of cell membranes (Lahti et al., 2005). Bai et al. (2016) observed a significant reduction in nitrate uptake into roots of cucumber in response to suboptimal root temperature. The positive effects of higher concentrations of N and K on plant growth disappeared when the root temperature was reduced from 20 to 12°C (Yan et al., 2013). In the 90-min experiments of Sago et al. (2011b), absorption rates of most ions in Welsh onion increased with root temperatures from 10 to 40°C. In the long term, shoot demand plays a key role in regulating the uptake rate of ions by physiological and morphological modification of the roots (Engels et al., 1992). A lower nutrient-absorbing surface area is the result of long-term cold acclimation. Yan et al. (2012) observed total N, P and K uptake of the cucumber seedlings at 10°C was reduced compared to 20°C for 30 days. However, the effects of root temperature on nutrient uptake vary with different physiological process, plant organs and ion types (Yan et al., 2012). For example, Engels (1993) found maize and wheat showed different K and P uptake rates at different root temperatures.

- Water uptake. Water absorption can be regulated by root temperature because water permeability of the root membrane is dependent on temperature (Sago et al., 2011a & b). Water uptake rates were reduced at low soil temperatures (Domisch et al., 2001). Altered root cell membrane structure and increased water viscosity are expected at lower root temperatures, contributing to the slow movement of water through the roots and subsequently lower water content in plant tissues (Kaufmann, 1975). Water

potential of leaf xylem was more negative for boreal trees when roots were exposed to 5 and 10°C compared with 20 and 25°C (Zhang & Dang, 2007). After reducing the root temperature of spinach from 20 to 5°C, the water potential and osmotic potential of leaf samples decreased significantly, and turgor pressure exhibited no differences (Chadirin et al., 2011). At root temperatures of 8, 10 and 14°C, root hydraulic conductivity and active transport of nutrients in cucumber plants were noticeably reduced (Lee, et al., 2004a & b). Root hydraulic conductivity of spinach grown at 5°C was only half of that noted at 20°C (Fennell & Markhart, 1998).

- Nutrient translocation. Translocation rates of the nutrients were indirectly regulated by internal nutrient demand (Engels & Marschner, 1992). For example, higher root temperatures promoted nutrient transport from root to shoot, resulting in increased nutrient concentrations in shoots, which may be due to the higher growth requirement of the shoot (Yan et al., 2012). In addition, the allocation of carbon to the root is favoured at high root temperatures due to the higher growth rates of root (Lahti et al., 2005). At lower root temperature, Aidoo et al. (2017) and Lloyd et al. (2011) found that more nitrogen was allocated to the roots for the potential growth of root to survive the low temperature stress. However, the carbon translocation rate to the roots was reduced probably due to a reduction in cell extension, which leads to the accumulation of carbon in the shoot (Lahti et al., 2005).
- Hormone signalling. Root temperature influences root-sourced signals via the xylem to regulate shoot growth (Dodd, 2005), such as cytokinin and gibberellin, which were significantly reduced as the root temperature of maize was reduced to 8°C for 17 days. Bai et al. (2016) further demonstrated that gibberellin acid homeostasis of cucumber was disrupted by a suboptimal root temperature of 16°C, which led to inhibition of root growth and nitrate uptake. The biosynthesis of abscisic acid

regulates stomatal closure, which affects the photosynthesis rate. The production of ethylene in the root zone was increased when the roots were under heat stress since ethylene inhibits stem elongation, leaf expansion and photosynthesis (Choong et al., 2016). Gibberellin acid is believed to play a key role in regulating plant growth in a changing environment, especially changes in ambient temperatures (Achard et al., 2008). The disruption of gibberellin acid biosynthesis led to suppression of root growth in *Arabidopsis* (Achard et al., 2008).

1.1.3 Food quality

Instead of the focus on increasing production, consumer awareness of food quality of fruit and vegetables has risen dramatically. Food quality is not a precise but a flexible term that comprises a variety of factors. According to ISO900 international standards, "*Food quality* is a total sum of features, characteristics and properties of a product, which bear on its ability to satisfy stated or implied needs". The overall quality of a food product is traditionally divided into three accepted categories: suitability value, sensory value and health value (Leitzmann, 1993). For horticultural crops, the perception of quality evaluated by the consumers includes four aspects: appearance, flavour, nutritive value, and safety. Among these factors, the appearances/conditions and flavour of fresh fruits and vegetables are the most cited concerns of consumers (Tronstad, 1995). Detailed factors in each aspect are listed in Figure 1.1. Some factors influence the appearance and flavour simultaneously, for example, freshness and ripeness, which are components of the appearance and are indicative of the expected flavour and aroma. In general, fruits and vegetables are a good source of minerals, vitamins and certain biologically active compounds needed to satisfy daily requirements (Camelo 2004). Currently, the attention of consumers is gradually switching to these nutritive values. In addition to minerals and vitamins, phytochemical compounds or

their metabolites of fruits and vegetables opened a new stage in nutrition science (Del Río-Celestino and Font 2020). For example, phenols, lignans and thiols are considered as antioxidants or neutralizers of free and anti-cancer compounds and are therefore beneficial to health (Slavin and Lloyd 2012).

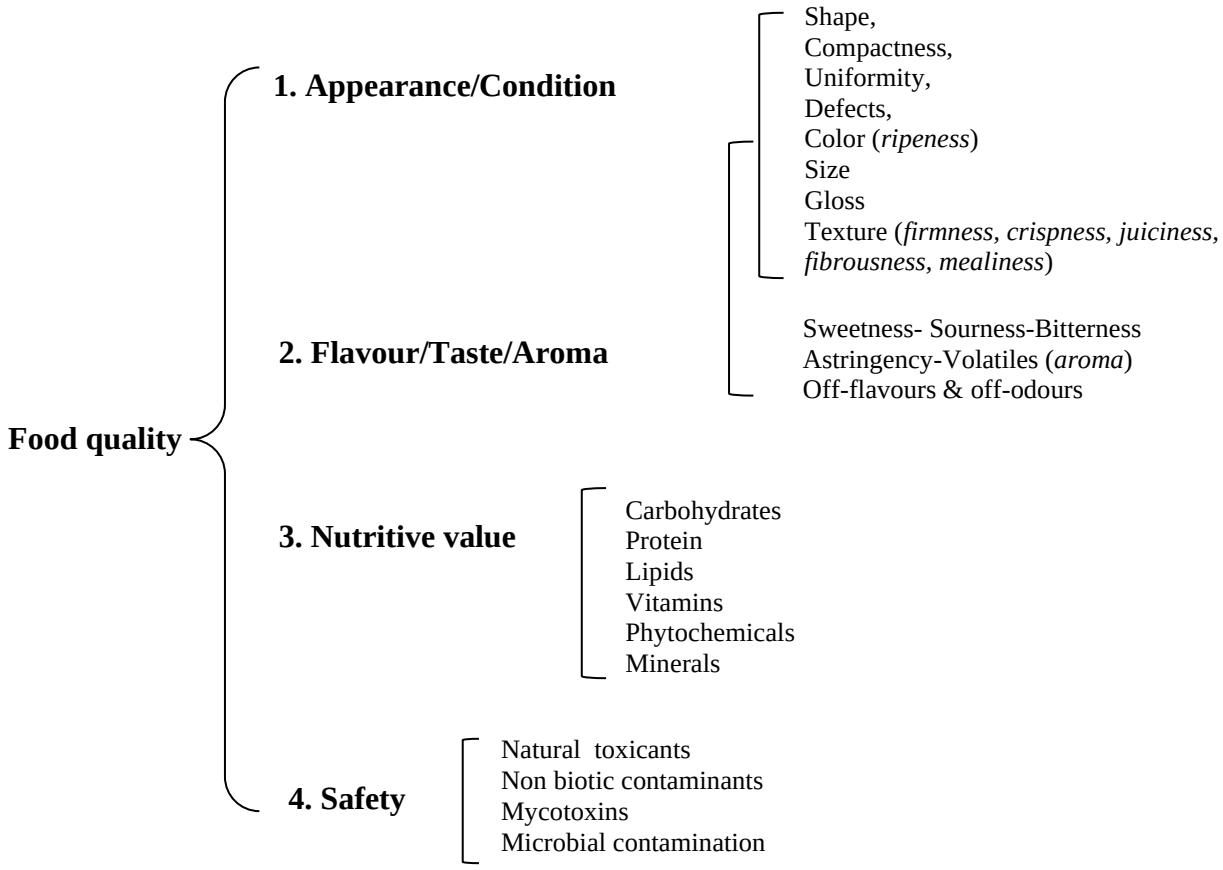

Figure 1.1 Consumer perception of quality (adapted from Camelo (2004))

The actual components of food quality are affected by the conditions of cultivation, varieties, climate and preparations (Poiroux-Gonord et al. 2010). As one of the important factors of the surrounding climate, root temperature influences certain quality characters of horticultural crops. Some of the studies have been summarized and listed in Table 1.1.

1.1.3.1 Flavour

For fruits and vegetables, flavour or taste is generally derived from the combination of sweetness and sourness (Kader 2008). Soluble sugars, such as glucose and fructose, are important components of sweetness, which directly influence consumer acceptance of fruits and vegetables (Malundo et al. 1995). Accumulation of soluble sugar in the shoot is regarded as a protection mechanism of plants against abiotic and biotic stress because soluble sugars are regarded as osmotic regulators, nutrient reservoirs and quasi-antioxidants under stress conditions (Sami et al., 2016). At sub-optimal root temperatures, the increased amount of sugar accumulation in the shoot is the result of reduced demand for assimilates from the sink because in addition to source supply, sink strength, growth rate and root respiration are also the main regulating processes of carbon distribution (Morison & Lawlor, 1999).

Organic acids (citric and malic acids) represent other essential components of flavour (Kader 2008). The ratio of sugar to organic acids decides the overall flavour of the products (Malundo et al. 2001). The total organic acid concentration is positively related with the total acidity, which is measured by titratable acidity. However, total acidity was less controlled by environmental factors than genetic traits (Shaw, 1990). Fujimura et al. (2012) found that the concentrations of malic acid and citric acid were not influenced by root cooling regardless of the cultivar or the season. Therefore, the influence of root temperature on flavour mainly depends on the adjustment of the sweetness.

1.1.3.2 Nutritive values

Elements of fruits and vegetables play a major role in providing the essential minerals of the human diet (Slavin and Lloyd 2012). Root temperature influences the uptake and translocation of mineral nutrients by modifying ion carrier enzyme activity, root cell

membranes, limited ATP energy produced through the root respiratory process and sink strength (Lahti et al., 2005; Yan et al., 2012).

Antioxidants of fruits and vegetables mainly consist of secondary metabolites. When plants are subject to biotic and abiotic stress, the accompanying generation and activity of reactive oxygen species (ROS) in plants are necessary responses to mediate numerous normal physiological activities (Sharma et al., 2012). Excessive ROS, such as superoxide anion radical, hydrogen peroxide and hydroxyl radical, cause damage to cells by modifying membranes, protein oxidation and DNA damage (Sharma et al., 2012). To dissipate toxic ROS, plants use enzymatic and non-enzymatic systems to reduce oxidative stress and protect the cells. Superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) are the three key enzymes involved in enzymatic systems of scavenging ROS (Zhang et al., 2007). In non-enzymatic protection systems, secondary metabolites, such as phenolic compounds, carotenoids, flavonoids, and ascorbic acids, play a major role in reactive species scavenging. These secondary metabolites are therefore largely affected by various environmental factors, including light, air temperature, CO_2 concentration, and root environment (Poiroux-Gonord et al. 2010). Sub- or supra-optimal root temperature can act as an unfavourable factor causing the generation of ROS, which leads to the upregulation of secondary metabolites in plants (Apel & Hirt, 2004). Since these secondary metabolites are important antioxidants and anti-cancer components in the human diet, enhancement of these compounds in the aim of producing value-added vegetables, fruits or medicinal plants is desired.

1.1.3.3 Safety

One of the main concerns for consumers about food safety of fruits and vegetables is the presence of pesticide residue (Dasika et al. 2012). Horticultural crops are traditionally regarded as a healthy food that provides essential nutrients for humans. However, in leafy

vegetables, harmful substances, such as nitrates and oxalates, are also present and produced by the crop itself (Jaworska, 2005; Kawazu et al., 2003). The accumulation of these compounds depends on plant species, cultivar, fertilization, light condition, temperature, soil condition, etc (Jaworska, 2005). Low root temperature stress is expected to depress the accumulation of nitrate and oxalate induced by the decreased root hydraulic conductance and a shortage of nitrogen in the shoots (Chadirin et al., 2011). Oxalate concentrations are highly correlated with total cation content; therefore, accumulation of excess cations is likely to result in the accumulation of high concentrations of oxalate (Kipnis & Dabush, 1988). At sub- or supra- root temperatures, the fraction of cations in the shoots tends to be reduced, which is subsequently expected to reduce the accumulation of oxalate.

Table 1.1 List of references about effects of root temperatures on bioactive components and minerals of fruits, vegetables and medical plants.

Root temperature treatment	Duration	Species	Compounds	Plant tissue	Effects	References
12, 20°C	3 weeks	Cucumber seedlings (*Cucumis sativus* L.)	Soluble sugar	Leaves	Low root temperature 12°C had significantly higher soluble sugar content than those at 20°C.	(Yan et al., 2013)
12, 25, 30°C	2 days	*Catharanthus roseus*, *Nicotiana tabacum*	Alkaloids: ajmalicine, catharanthine, nicotine	Leaves and roots	Root temperature of 12 °C enhanced the root ajmalicine, catharanthine content than control group 25°C.	(Malik et al., 2013)
10, 25, 30°C	7 days	Red leaf lettuce (*Lactuca sativa* L. cv. Red Wave)	Anthocyanin, phenols, sugar and nitrate	Leaves	Under low root temperature 10°C, leaves contained a higher concentration of anthocyanin, phenols, sugar and nitrate than other temperatures.	(Sakamoto and Suzuki, 2015a)
20, 25, 29, 33°C	7 days	Carrot seeds (*Daucus carota* L. cv Tokinashigosun)	Total phenolic compounds, soluble solid content, chlorophyll, carotenoids, carotene, anthocyanin	Tap roots and leaves	Total phenolic compounds and soluble solid content increased in tap roots and chlorophyll content reduced in carrot leaves under high root temperature treatment (33°C). No differences were found in total carotenoids, carotenes, and anthocyanin content in tap roots among the different temperature treatment groups.	(Sakamoto and Suzuki, 2015b)
20, 25, 30°C	21 days	African snake tomato (*Trichosanthes cucumerina* L. Cucurbitaceae)	Phenolics, ascorbic acid, chlorophylls, Ca, Mg, P, K, Fe and Mn	Roots, stems and leaves	The amounts of phenolic, ascorbic acids and chlorophyll increased as the root temperature increased. Higher amounts of Ca and K were present in the root at lower root temperature (20°C).	(Adebooye et al., 2010)
5, 20°C	7 days or 14 days	Spinach (*Spinacia oleracea* L. cv. Orai)	Sugars, ascorbic acid, Fe, nitrates, oxalic acid	Shoots	Sugars, ascorbic acid and Fe were significantly enriched in edible shoots, while the concentration of nitrates and oxalic acids were decreased at low root temperature of 5°C.	(Chadirin et al., 2011)
35, 45, 55°C	2 days	*Radix Scutellariae*	Flavonoids: Baicalin, wogonoside, baicalein	Roots	Baicalin, wogonoside and baicalein concentration increased when the fresh roots were exposed to 55°C for 2 days.	(Fu et al., 2017)
20, 25, 30°C	4 weeks	*E. sativa*	Phenolic compounds, K, Mg, Ca and Fe	Shoots and roots	At root temperature 20°C, K and Ca contents were highest, but with the lowest total phenolics content.	(He et al., 2016)
20, 25, 30°C	19 days	Coriander (*Coriandrum sativum*)	Total phenolic compounds, chlorogenic acid, rutin, trans-2-decenal	Leaves and stems	Content of total phenolic, trans-2-decenal, chlorogenic acid, were highest at root temperature 30°C .	(Nguyen et al., 2019)
10, 20, 28, and 36 ◦C	25 days	*Agastache rugosa*	Rosmarinic acid, tilianin, acacetin, chlorophyll	Leaves, roots, flowers, and stems	28 °C Root temperature produced the greatest accumulation of rosmarinic acid and tilianin contents.	(Lam et al., 2020)

1.2 Horticultural model crops

Cocktail tomato (*Solanum lycopersicum*) and Chinese broccoli (*Brassica oleracea* var. *alboglabra* Bailey) were chosen as our model plants to study their growth and food quality after different root temperature treatments. Tomato is chosen as the model plant because it is the second most important vegetable crop (FAO, 2019) and ideal for studying the quality and ripening of fleshy fruits due to its relatively short generative time (Bertin & Génard, 2018). Chinese broccoli was chosen based on its fast-growing properties and increasing popularity among consumers.

1.2.1 Cocktail tomato

Tomatoes are important agricultural commodities worldwide. According to the data from FAO (2019), the world production quantity of tomatoes has gradually increased since 1980 and yielded more than 180 million tonnes in 2017. The tomato fruit consists of skin, pericarp, and locular contents. The local cavities are filled with jelly-like parenchyma cells that surround the seeds. Cocktail tomatoes are small-sized fruits with intense bright red color and sweet flavor (Campos Menezes, 2012). Given its tastier and attractive characters compared with normal-sized cultivars, the growth and sales of cocktail tomatoes are increasing worldwide (Sonntag et al., 2019). The good taste and bright color of cocktail tomatoes are attributed to the higher concentration of sugars, acids and carotenoids (Figàs et al., 2015). This higher level is attributed to the greater skin to volume ratio of cocktail tomatoes because most of these compounds accumulate in the outer skin of tomatoes (Stewart et al., 2000). Another explanation for the higher concentration values is the lower fruit size and yield per plant in cherry or cocktail tomatoes compared with regular-sized tomatoes (Panthee et al., 2013).

1.2.2. 1 Nutrients value

The overall flavor of tomato is largely determined by the concentration of sugars and acids (Kader, 2008). The main sugars present in tomatoes are glucose and fructose at an approximately equal ratio (Beckles, 2012). Sucrose exists in low levels or cannot be detected in the current cultivated tomatoes (Beauvoit et al., 2014). Citric acid and malic acid are the main organic acids of tomato fruits (Siddiqui et al., 2015). Tomato fruits also supply essential elements for human health, such as K, Ca, P and Mg (Vicente et al., 2009).

In addition to essential mineral elements, tomatoes and tomato-based products contain high levels of carotenoids, providing precursors for biosynthesis of vitamin A. Carotenoids are typically a class of 40-carbon hydrocarbon compounds with an isoprenoid backbone (Story et al., 2010). Carotenoids produce plant colors via orange, red, and yellow pigments that are synthesized by the general isoprenoid biosynthetic pathway.

Lycopene is the predominant carotenoid in tomatoes; therefore, the characteristic deep-red color of ripe tomato fruit is mainly due to lycopene (Siddiqui et al., 2015). Lycopene is a terpenoid with 13 double bonds, 11 of which are conjugated, and these double bonds are naturally localized in the pericarp of tomato fruits (Viuda-Martos et al., 2014). During the maturation stage of tomato as the color changes from green to red, chlorophyll is gradually degraded, and lycopene is biosynthesized and becomes the main pigment (Ilahy et al., 2011). Lycopene is not a provitamin A carotenoid but is ranked as the most potent antioxidant among carotenoids within the tomato fruits (Viuda-Martos et al., 2014). Recent studies have demonstrated that the consumption of lycopene-rich foods is associated with reduced risks of certain cancers and some cardiovascular diseases (Böhm, 2012; Kelkel et al., 2011; Kong et al., 2010). Tomatoes have been reported to contain on average 30 mg lycopene per kg raw materials, and a higher concentration of 60 mg/kg is noted in cherry and cocktail

tomatoes (Adalid et al., 2010). The amount of lycopene in fresh tomato fruit was strongly influenced by variety, maturity, cultural practices and environmental factors (Erge & Karadeniz, 2011; Ilahy et al., 2011; Toor et al., 2006).

Although present at lower concentrations than lycopene, β-carotene is also abundant in tomato fruits (Cortés-Olmos et al., 2014). β-carotene is also classified as a terpenoid with a beta-ring at both ends and exhibits prominent provitamin A activity (Fernández-García et al., 2012), which is related to normal development of vision (Valtueña et al., 2011). Moreover, β-carotene is a potent antioxidant against some types of cancer and age-related macular degeneration (Wang et al., 2010). Similarly, the concentration of β-carotene is largely determined by fruit color, ripening stage, cultivars and environmental factors (Erge & Karadeniz, 2011; Flores et al., 2017; Hdider et al., 2013).

In addition to carotenoids, ascorbic acid (Vitamin C) is another source of antioxidant compounds in tomato fruits and plays a crucial role in scavenging of ROS generated after exposure of plants to stress. Among the antioxidants of tomatoes, ascorbic acid is the most efficient (Kotíková et al., 2011). As a cofactor for several important enzymes, ascorbic acid is also important in plant development and hormone signaling (Mellidou & Keulemans, 2012). Dietary intake of ascorbic acid has also long been correlated with a decreased rate of several cardiovascular diseases (Raiola et al., 2014). The concentration of ascorbic acid in tomatoes is low compared to other high ascorbic acid fruits and vegetables, but tomatoes remain the main source of dietary intake (George et al., 2004). The concentration of ascorbic acid is influenced by different genotypes, agricultural practices, and environmental factors.

1.2.2.2 Effects of temperature on tomato

Tomatoes originated from subtropical areas and are sensitive to low temperatures. No growth would be expected if the temperature is below 12°C. In general, the optimum air temperature for tomato production is 21-25°C with an average monthly minimum temperature above 18°C and a monthly maximum temperature below 27°C (Araki et al., 2000). Lower temperatures between 18 and 20°C promote fruit setting by increasing the pollen quality (de Koning, 1994). During the fruit development stage, air temperatures less than 16°C can cause flower abscission, whereas temperatures greater than 30°C cause cracked fruit and blotchy ripening. Furthermore, temperature significantly affects the partitioning of assimilates between the vegetative and generative portions of the tomato. High air temperature between anthesis and fruit ripening cause the initial trusses to appear faster and more fruits on the plant at the expense of vegetative growth, which is attributed to a delay in later truss (Van Ploeg & Heuvelink, 2005). In addition to growth, Krumbein et al. (2012) found out that air temperature greater than 20°C is optimal for lycopene production, but lower air temperatures at 15°C seem to inhibit the biosynthesis of lycopene during ripening.

Root temperature has long been recognized as an important factor for tomato plant growth. The cultivated tomato *Lycopersicon esculentum* is vulnerable to low root temperatures (Bloom et al., 2004; He et al., 2014). The mechanisms of root temperature on tomato plant growth are the results of both direct and indirect processes. Root growth and nutrient and water uptake are directly influenced, whereas stomatal conductance, leaf expansion, photosynthesis, hormone synthesis and distribution are indirectly related (Kawasaki et al., 2013; Kawasaki et al., 2014; Gonzalez-Fuentes et al., 2016). Cooper (1973) described the general response curve of all species to root temperature as a downward parabola, with optimal root temperature for tomatoes between 25 and 30°C. Tomato plants at

different growth stages have different root temperature preferences: 20-30°C for vegetative growth (Fujishige & Sugiyama, 1968; Fujishige et al., 1991), 25-30°C during flower differentiation, and 15-30°C during fruit development (Fujishige et al., 1991). Root temperature also influences the distribution of carbohydrates between shoots and roots by modifying the sink strength of roots (Hurewitz & Janes, 1983; He et al., 2009; Islam et al., 2011). The shoot to root ratio of tomato is positively correlated with root temperature (Gosselin & Trudel, 1982). In addition to the influence on growth, many studies have proved that root temperature influenced the sugar concentrations of different plant organs. For example, lower root temperature induced higher sugar concentrations in the leaves of red leaf lettuce (Sakamoto & Suzuki, 2015a), spinach (Hidaka et al., 2007) and tomato fruits (Fujimura et al., 2012). Lower root temperature was also demonstrated recently to enhance the accumulation of antioxidants, such as β-carotene and Vitamin C, in hydroponically grown carrots and spinach (Sakamoto & Suzuki, 2015b; Hidaka et al., 2007). However, information is still lacking on the mechanism by which root temperature influences the concentration of carotenoids, Vitamin C and essential elements in cocktail tomatoes.

1.2.2 Chinese broccoli

Brassica vegetables are a family of important vegetable species consumed around the world. Chinese kale belongs to the same species as common kale (*Brassica oleracea*) but is in the cultivar group *alboglabra*. Chinese kale originated from China and is also known as Chinese broccoli, Chinese kale, Kailan, or Gai lan (Hanson et al., 2011). Chinese broccoli is mainly distributed in South China and was recently introduced to Southeast Asia, Japan, Europe, America and Australia (Lei et al., 2017). Chinese broccoli cultivars in South China can be classified based on different criteria. Chinese broccoli can be categorized into two types according to the petal color: yellow or white (Lei et al., 2017). The Latin name

“Bailey” refers to the white petal variety, which is the most common type (Lei et al., 2017). Chinese broccoli can be classified into three groups based on the growth period: early-, mid- and late-maturity cultivars. Low air temperature is not necessary for bolting in early-maturity cultivars but is critical for late-maturity cultivars.

Chinese broccoli is an annual plant, which develops blue-green lustrous leaves, thick crispy stems, yellow or white flower buds (Okuda et al., 2000). Leaves exhibit an alternating pattern with a long petiole, and the shape is dependent on different cultivars, exhibiting long oval, round and nearly round shape (Cao, 2004). The roots of Chinese broccoli are shallow and concentrated within 15-20 cm under the soil surface, and the root system includes a taproot and numerous lateral roots (Cao, 2004). The resurgence capability of the root system is relatively strong, and adventitious roots are easily developed (Cao, 2004). The crop is harvested at the stage after the appearance of inflorescences but before flowering, and the stems are tender (Hanson et al., 2011). The commercial harvest criterion is when the height of bolting stalk is the same as apical leaves (Sun et al., 2011).

1.2.3.1 Nutrition value

Chinese kale is usually grown for its bolting stems, which is a common edible part, whereas tender young leaves are also widely consumed as leafy vegetables (Yin et al., 2015). The bolting stem contains 92-93 g water per 100 g fresh weight; therefore, it is tender and crispy with good flavor (Cao, 2004). The flower stalk exhibits high nutritional value given its richness in vitamins, minerals, antioxidants and anticarcinogenic compounds, such as vitamin C, carotenoids and glucosinolates (Sun et al., 2011).

Glucosinolates are a group of nitrogen- and sulfur-containing secondary metabolites, sharing a common chemical structure consisting of a β-D-thioglucose and sulphonated oxime

moieties plus a variable side chain derived from amino acids (Fahey et al., 2012). Based on variations of the side-chain, glucosinolates are classified into three groups: aliphatic, aromatic and indolic (Sønderby et al., 2010). Plants containing glucosinolates possess the endogenous enzyme myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1), which is localized in myrosin cells and separated with glucosinolates spatially (Ishida et al., 2014). When glucosinolate-containing cells are mechanically damaged or attacked by pathogens, glucosinolates are hydrolyzed by myrosinase in the presence of water into an aglycone moiety, glucose and sulfate (Wang et al., 2011). The aglycone moiety is unstable and rearranges to form isothiocyanates, thiocyanates, nitriles, etc., upon the structure of glucosinolates and the reaction conditions (Kissen et al., 2009). The hydrolysis products are important pungent compounds that affect the flavour, taste and acceptance of Brassicaceae vegetables (Wang et al., 2011). Some isothiocyanates products, such as indole-3-carbinol and sulforaphane, have been found to exhibit strong anticarcinogenic properties (Choi et al., 2010). Therefore, research about glucosinolates has been in the spotlight.

Cruciferous vegetables are rich in glucosinolates (Padilla et al., 2007), and Chinese broccoli contains 6.5-7.5 mg/g dry weight of total glucosinolates, which is greater compared with other Chinese Brassica vegetables (Chen et al., 2006). To date, 13 glucosinolates belonging to three groups have been identified in different plant tissue of Chinese broccoli (Table 1.2). The main group of glucosinolates discovered in Chinese broccoli are aliphatic, representing over 70% of glucosinolates both in the leaves and bolting stems, followed by indolic and aromatic glucosinolates (Qian et al., 2016; Sun et al., 2011). Gluconapin and glucoraphanin are the two most abundant glucosinolates existing in leaves and bolting stems (La et al., 2009; Sun et al., 2011& 2012 a). The enzymatic product of glucoraphanin, sulforaphane, has been the focus of many studies and is the strongest natural anti-carcinogenic substance (Cheung & Kong, 2010). Recent studies have shown intake of

sulforaphane supplements could alleviate the syndrome of autism spectrum disorder (Singh et al., 2014). Moreover, sulforaphane is an indirect antioxidant, which induces many cytoprotective proteins, including antioxidant enzymes (Guerrero-Beltrán et al., 2012). Phenethyl isothiocyanate, hydrolysis products of gluconasturtiin, is also a potent chemopreventive agent via regulation of diverse molecular mechanisms (Gupta et al., 2014).

Total and individual glucosinolate content indicated significant differences among different genotypes and plant organs (La et al., 2009; Sun et al., 2011& 2012 a). In addition to genetic characteristics, glucosinolate levels are also affected by preharvest factors, including climatic factors, nutrient availability and agronomic practices. La et al. (2009) found out that total glucosinolates concentration in the Chinese kale bolting stem decreased after increasing N levels from 100 to 200 mg/L and then decreased from 200 to 400 mg/L. Elevated CO_2 concentrations increased the total glucosinolates concentration in bolting stems of Chinese broccoli, but this effect was also dependent on the nitrogen concentration in the soil (La et al., 2009). Chinese kale sprouts treated with red light before harvest inhibited the degradation of glucosinolates during storage (Deng et al., 2017). Application of NaCl to the seeds of broccoli altered the synthesis of total and individual glucosinolates (Guo et al., 2013). Therefore, glucosinolate concentration depends largely on environmental factors during growth.

Table 1.2 List of glucosinolates found in different plant tissue of Chinese broccoli.

Group	Trivial name	Chemical names of R-groups	Side-chain structure	Plant tissue of Chinese broccoli (+ present; -absent)			
				Sprouts	Rosette leaves	Bolting stems	Mature leaves
Aliphatic	Glucoerucin	4-Methylthiobutyl	CH_3-S-$(CH_2)_4$-	+	+	+	-
	Glucoraphanin	4-Methylsulfinylbutyl	CH_3-SO-$(CH_2)_4$-	+	+	+	+
	Glucoalyssin	5-Methylsulphinylpentyl	CH_3-SO-$(CH_2)_5$-	-	-	+	-
	Sinigrin	2-Propenyl	CH_2=CH-CH_2-	+	+	+	+
	Gluconapin	3-Butenyl	CH_2=CH-$(CH_2)_2$-	+	+	+	+
	Glucobrassicanapin	4-Pentenyl	CH_2=CH-$(CH_2)_3$-	-	-	+	+
	Progoitrin	2-(R)-2-Hydroxy-3-butenyl	CH_2=CHCH(OH)CH_2-	+	+	+	+
	Glucoiberin	3-Methylsulphinylpropyl	CH_3-SO-$(CH_2)_3$-	+	+	+	+
Indolic	4-Hydroxy glucobrassicin	4-Hydroxy-3-indolylmethyl	Indole-4-OH-3-CH_2-	+	+	+	+
	Glucobrassicin	3-Indolylmethyl	Indole-3-CH_2-	+	+	+	+
	4-Methoxy glucobrassicin	4-Methoxy-3-indolylmethy	Indole-4-OCH_3-3-CH_2-	+	+	+	+
	Neoglucobrassicin	1-Methoxy-3-indolylmethyl	Indole(OCH_3)-3-CH_2-	+	+	+	+
Aromatic	Gluconasturtiin	2-Phenylethyl	Benzene-$(CH_2)_2$-	+	+	+	-

Sprouts: harvested from seedlings of 10-day old; rosette leaves: harvested from plants with 8-10 leaves before bolting; mature leaves: harvested with bolting stems after bolting (Chen, 2006; La et al., 2009 & 2011; Sun et al., 2011).

Extraction of glucosinolates is the most important step before analysis. Several extraction methods have been developed to deactivate myrosinase before tissue disruption. Boiling the plant tissue in 70% methanol (v/v) at 75°C is the most widely used method based on ISO 9167-1. However, hot methanol vapor is hazardous and time-consuming. High boiling temperature also degrades some indole glucosinolates, such as 4-hydroxy-glucobrassicin and 4-methoxyglucobrassicin (Oerlemans et al., 2006). An alternative simple method involved the use of a high concentration of cold methanol (80%) to denature myrosinase activity and prevent the hydrolysis of glucosinolates (Doheny-Adams et al., 2017). This simplified method is applied to most glucosinolates extractions from different Brassica plants tissue. The method involves less cost and time but offers comparable

glucosinolates extraction efficiency compared to the ISO method (Doheny-Adams et al., 2017).

1.2.3.2 Effects of temperature on Chinese broccoli

Chinese broccoli is a cool-season crop, and the optimum temperature for rapid growth is 18-28ºC (Kopta & Pokluda, 2010). In addition, Chinese broccoli exhibits frost tolerance. At different growth stages, different temperature requirements are noted. During the adaptation stage after transplanting, the optimal temperature combinations for the plants are 25-26ºC during day and 16-17ºC in the evening. Before bolting, decreasing air temperature has been found to have a promoting effect on bolting and flower stalk differentiation and subsequently the yield and quality (Yang & Yang, 2002). High temperatures (38/26°C) decrease the yield of Chinese broccoli but improve chlorophyll, vitamin C and sugar concentrations in bolting stems (Chen et al., 2006).

In addition to growth, it is widely recognized that the concentration and composition of glucosinolates in plants are substantially influenced by both genetic and environmental factors (Mithen et al., 2010). Studies have assessed the effects of air temperature on the concentration of glucosinolates in other Brassica vegetables. The accumulation of aliphatic and indolic glucosinolates in leaves and stems of wild cabbage was enhanced by increased air temperature exposure at 32°C compared to 12°C (Charron & Sams, 2004). Justen & Fritz (2013) described that high temperature treatments increased total and individual glucosinolate concentrations both in roots and shoots of two turnips cultivars. Pereira et al. (2002) indicated that both high and low air temperature stress promoted total glucosinolates biosynthesis. Moreover, the concentration of the aromatic glucosinolate gluconasturtiin was inversely correlated with high temperature. Another study by Engelen-Eigles et al. (2006) in watercress (*Nasturtium officinale*) also proved that the level of gluconasturtiin was increased at 10 and

15ºC compared to 20 and 25ºC. In addition to air temperature, root temperature is also a critical factor of microclimate in hydroponic conditions. Elevated soil temperatures increase glucosinolate levels in *Brassica oleracea* (Del Carmen Martínez-Ballesta et al., 2013). However, research about the effects of root temperature on glucosinolates remains incomplete.

1.3 Objectives and hypothesis

The main purpose of the study was to determine the effects of root cooling (10°C) in the greenhouse under normal production conditions on the quality parameters of horticultural crops. For this purpose, two cocktail tomato cultivars and one Chinese broccoli cultivar were studied. Considering the potential negative effects of root cooling on biomass and yield, root cooling treatment was applied after the second inflorescence of cocktail tomatoes and shortened to one week before harvest in Chinese broccoli with the aim of minimizing the negative effect. The hypothesis was that root cooling could improve some quality parameters (sugar, antioxidants and minerals) of these two horticultural crops with a minimum reduction in the yield.

1.4 Thesis outline

Chapters 2-3 in this thesis refer to publications that have already been published by scientific journals or have been prepared for submission.

The first paper (He et al., 2019), which is described in chapter 2, addresses the effects of root temperature on food quality of two cocktail tomatoes. We assessed the impact of root cooling on plant growth and fruit quality of two cocktail tomato cultivars (*Lycopersicon esculentum* cv 'Amoroso' and cv 'Delioso') during the winter of 2017-2018 and the summer of

2018. Since cocktail tomatoes are becoming more popular among consumers, we chose two newly bred high lycopene cultivars from the Netherlands. In the first preliminary experiment, we set the root temperature after transplanting at 10 and 15°C and observed that the growth of young seedlings with 10°C roots was strongly inhibited. Considering the negative effect on growth and subsequent effect on the biomass, we decided to start root cooling during the generative stage. In the second preliminary experiment, 15 and 20°C root temperatures were started after the second inflorescence, and the plants did not show any differences in tomato biomass and size. Considering the minor effects, we decided to reduce the root temperature to 10°C in the cool group, and the other group without treatment was regarded as control. The results showed that root cooling could improve the overall qualities of the cocktail tomatoes, and the reduction in the yield depends on the cultivar and season.

The second paper (He et al., 2020), which is described in chapter 3, deals with Chinese broccoli under different root temperatures for a longer period. Root temperature treatment started two days after transplanting until the harvest. Based on the experiences of cocktail tomatoes, we used 15 and 20°C root temperatures to cultivate the Chinese broccoli hydroponically. The yield was not affected, and the quality of various factors, such as glucosinolates, was also not enhanced. Therefore, we reduced the root temperature to 10°C in the 2nd experiment. The results indicated significant improvement in the quality (soluble sugars and glucosinolates), but the yield was reduced accordingly. However, the results of the first two experiments showed that root cooling could influence key components of Chinese broccoli.

The third manuscript (He et al., 2021), which is described in chapter 4, deals with Chinese broccoli under two root temperatures for a short period. We investigated whether one week of root cooling before harvest could improve food quality (soluble sugars, total chlorophyll, glucosinolates, and minerals) of Chinese broccoli in the summer of 2018 and

autumn of 2019 without significantly reducing the yield. Based on the results of the first two experiments, the treatment duration was shortened to one week before harvest. The final results were quite promising compared to previous results. The reduction of the yield is highly alleviated, but this effect also depends on ambient temperature and light.

In chapter 5, the sensory evaluations of two cocktail tomatoes under the effects of root temperatures are described. Sensory evaluation was conducted as a supplement to previous chemical analysis and the first trial without trained and professional panels given the missing capacity. In the first assessment, we adopted a descriptive analysis and let the volunteers describe the aroma, hardness, sweetness, sourness and overall using numbers from 1 to 5. The results did not show significant differences except for the aroma of “Delioso”, which was improved in the root cooling group. Therefore, we used preference testings in the second test, and the participants could vote for the preferred tomato. The results were distinctive and consistent with the previous chemical sugar and lycopene analysis, further demonstrating that root cooling could improve some qualities of cocktail tomatoes.

Chapter 2

Effects of root cooling on plant growth and fruit quality of cocktail tomato during two consecutive seasons

Based on a journal article published as He, F., Thiele, B., Watt, M., Kraska, T., Ulbrich, A., & Kuhn, A. J. (2019). Effects of root cooling on plant growth and fruit quality of cocktail tomato during two consecutive seasons. *Journal of Food Quality, 2019.* https://doi.org/10.1155/2019/3598172

2.1 Introduction

Tomato (*Lycopersicum esculentum* Mill.) is an important horticultural crop worldwide with an increasing area of production, reaching 4.8 million hectares with an average of 37.6 tonnes/hectares and an overall production of more than 18 million tonnes respectively in 2017 (FAO, 2019). Health-promoting effects as well as potential risk of tomatoes and tomato-based products consumption for humans are well known and have been reviewed by Salehi et al. (2019). Hence, the protective action is typically assigned to significant levels of antioxidants such as vitamin C (Borguini & Ferraz da Silva Torres, 2009), lycopene (Story et al., 2010), or carotenoids (Perera & Yen, 2007). Cocktail tomatoes with an average weight of 20-50g are perceived as tastier by consumers (Casals et al., 2019) and due to the suitable size they are getting more popular among consumers. Cocktail tomatoes are proven to contain higher levels of sugars, carotenoids and other antioxidants than normal sized ones (Leonardi et al., 2000), because of its higher skin to volume ratio (Stewart et al., 2000).

Among the environmental factors, temperature plays a crucial role in the growth of tomato plants and development of fruits. At sub-optimal air temperature for the vegetative stage, tomato seedlings tend to produce larger cells to store more starch, indicated thicker leaves and relative lower growth rate (Venema et al., 2008). Even short periods of low temperatures could induce blossom end scarring of fruits, making them sensitive to bruising and possible entrance for postharvest diseases (Barten et al., 1992). During the flower development stage, cooler air temperature induced an increase in the number of flowers, late ripeness and eventually larger fruits (Rylski, 1979; Sawhney & Polowick, 1985). The optimal air temperature for fruit setting is 18-20°C, and higher than 30°C causes fruit cracking and blotchy ripening (de Koning, 1994). During fruit development stages, accumulation of carotenoids is promoted above 10°C, but inhibited above 30°C air temperature (Dumas et al.,

2003; Gautier et al., 2008).

Besides air temperature, root temperature has long been recognized as an important factor for the growth of the tomato plant. Originating from tropical regions, the cultivated tomato, *Lycopersicon esculentum*, is vulnerable to low root temperature (Bloom et al., 2004; He et al., 2014). The mechanisms of root temperature on the growth of tomato plants are the results of both direct and indirect processes. Root growth, nutrient and water uptake are directly influenced, whereas stomatal conductance, leaf expansion, photosynthesis, hormone synthesis and distribution are indirectly related (Veselova et al., 2005; Malcolm et al., 2008; Ntatsi et al., 2013; Kawasaki et al., 2013 & 2014; Gonzalez-Fuentes et al., 2016). Cooper (1973) described the general response curve of all species to root temperature as a downward parabola, with optimal root temperature for tomato being between 25 and 30°C. Tomato plants at different growth stages have different root temperature preferences: 20-30°C for vegetative growth (Fujishige & Sugiyama, 1968; Fujishige et al., 1991), 25-30°C during flower differentiation, and 15-30°C during fruit development (Fujishige et al., 1991). Root temperature also influences the distribution of carbohydrates between shoot and root by modifying the sink strength of root (Hurewitz et al., 1983; He et al., 2009; Islam, 2011). The shoot to root ratio of tomato is positively correlated with root temperature (Gosselin & Trudel, 1982).

The overall flavor of tomato is largely determined by the concentration of sugars and acids (Kader, 2008). Many studies have proved that root temperature influenced the sugar concentrations of different plant organs. For example, lower root temperature induced higher concentration of sugars in the leaves of red leaf lettuce (Sakamoto & Suzuki, 2015a) and spinach (Hidaka et al., 2007). Carotenoids of tomatoes are an important source for human nutrition due to high frequency in the diet (Krumbein, et al., 2006). A number of

environmental factors, such as light intensity, CO_2 levels, salinity and temperature, are known to influence the levels of carotenoids in tomatoes (Krumbein et al., 2006; Gautier et al., 2008). Lower root temperature was also demonstrated recently to enhance the accumulation of carotenoids, such as β-carotene, in hydroponically grown carrots (Sakamoto & Suzuki, 2015b). Ascorbic acid (Vitamin C) is another important antioxidant of tomato fruits. One week application of 5°C root temperature to the root of spinach enriched the levels of ascorbic acids in leaves (Hidaka et al., 2007). Tomato fruits also supply essential elements for human health, such as K, Ca, P and Mg (Vicente et al., 2009). Root temperature has been shown to alter the uptake and translocation of minerals to different parts of plant, such as K and P of maize (Engels, 1993); Ca, Mg, P, K, Fe and Mn of African snake tomato (Adebooye et al., 2010); Fe of spinach (Hidaka & Yasutake, 2007); K, N, P, Ca, Mg of young tomato plants (Kawasaki, et al., 2014). Thus, proper manipulation of root temperature could improve the tasty and healthy components, leading to increased crop market value.

Maintaining the root temperature in the optimal range has been used as an energy-efficient method to alleviate injury caused by suboptimal air temperature (Kawasaki & Yoneda, 2019). Trudel & Gosselin (1982) and Gosselin &Trudel (1983) reported that root temperatures lower than 16°C greatly reduced the yield of tomato, while warming the roots partially alleviated cool air temperature in the night by showing a rise in yield. Kawasaki et al. (2014) also observed that root heating at low air temperature increased the root growth and total yield of tomato. Around 25°C root temperature increased photosynthesis, stomatal conductance and shoot growth at high air temperatures (40°C day/23°C night) (Nkansah & Ito, 1994). Furthermore, roots growth and nutrient uptake of young tomato plants were enhanced by root cooling at higher air temperatures by production of auxin (Kawasaki et al., 2013). However, little is known about the effect of excessive root cooling on plant growth and especially, fruit quality of tomato.

It has been shown that manipulation of drought stress at the late stages of plant development improved the overall fruit quality without reducing yield (Ripoll et al., 2014 & 2016). In line with the findings under water stress, application of root cooling after the 2nd anthesis only may, however, improve the fruit quality without decreasing the yield. We hypothesized that such conditions reduce root sink strength for photoassimilates and favor the translocation of carbohydrates to the growing fruits therefore. In addition, antioxidants, e.g. ascorbic acid and carotenoids, may be increased under suboptimal root temperature stress. Concentrations of ions are unaffected or enhanced after long-term adaptation to low root temperature by increasing the capacity for uptake and translocation (White et al., 1987). To test this hypothesis in cocktail tomato, a soilless culture (Rockwool) was carried out in our experiments in the two seasons (2017-2018 winter and 2018 summer) at the start of the 2nd flowering, two root temperature treatments were applied: 10°C and control. Plant and fruit growth, the concentrations of carbohydrates (glucose, fructose and sucrose), organic acids (malic acid, citric acid and ascorbic acid), carotenoids (lycopene and β-carotene), and elements (macro- and micro-) were measured after harvest.

2.2 Materials and Methods

2.2.1 Plant material and growth conditions

Cocktail tomatoes cv 'Delioso' and cv 'Amoroso' were provided from Rijk Zwaan breeding company (The Netherlands). Seeds were sown on 11th October 2017 and 10th April 2018 into Rockwool plugs (25×25×40mm with a 6/16 mm hole, Grodan Vital, Roermond, The Netherlands), which were previously submerged in distilled water for one hour. All the plugs with seeds were put in the tray and covered with a lid to prevent light and temperature was kept at 25ºC. At 3-4 DAS (Days after sowing), the seeds were germinated and the lid was

removed. After the 1st true leaf was developed (around 15 DAS), seedlings were transferred to Rockwool cubes (100 ×100×65mm, Grodan Vital, Roermond, The Netherlands) and fertilized with half-strength Hoagland solution (mg/L): N(105.0), Ca (100.2), K (117.3), Mg (24.6), S(32.0), P (15.5), Fe (0.5), Mn (0.55), Cu (0.064), Zn (0.065), B (0.54), Mo (0.048) with EC 1.2 dS/m, pH 6.0. When the roots reached the bottom of the cubes (38-44 DAS), about 3 to 4 true leaves, the seedlings were placed on the top of the Rockwool slabs (1000×200×75mm; Grodan Vital, Roermond, The Netherlands). The composition of the nutrient solution was changed as follows (mg/L): N (120.6), Ca (108.3), K (180.6), Mg (28.8), S (70.5), P (23.8), Mn (0.27), Zn (0.16), B (0.04), Cu (0.025), Mo (0.023) and Fe (0.419) with pH around 5.8, EC 2.8. The nutrient solution was supplied automatically every hour from 6.00 until 19.00 and the total amount was 2-4 L per day per plant in order to keep 30-40% efflux and reduce salt accumulation (Wu & Kubota, 2008). Plants of both seasons were trained to one stem high wire system. In both seasons, plants were grown at a density of 2.5 m^2 (0.5 m between and 0.5 m within-row). Side shoots were pruned regularly and leaves were removed once they were below the cluster that was picked. All the plants were topped, leaving two leaves above the 7th cluster. Flowers at anthesis were vibrated by electronic toothbrush to stimulate pollination. Six supplementary high pressure discharge lamps (720 µmol/s) (MGR-K400, DHLICHT, Germany) were open 16h from 6.00 to 22.00 in the two seasons to compensate for low daily PARs. Daily maximum air temperature and irradiation were continuously measured and recorded during both seasons by the climate sensor in the middle of the greenhouse.

2.2.2 Experimental design and root temperature management

In both seasons, two cultivars were randomly located on 14 slabs in two rows, with 2 plants in each slab. Considering the border effect, four plants in the corner of two rows were

excluded from measurement. There were two treatments, control and roots treated at 10°C root temperature. Cooling mats (Clina Heiz und Kühlelemente GmbH, Germany) circulated with cooled distilled water from thermostat (Julabo, Germany) were placed on the top and bottom of slabs. Thermal insulation mats were wrapped outside the cooling mats to reduce heat transfer between Rockwool and ambient air. The temperature of thermostat was set at 10°C and ten temperature loggers (developed by IBG-2, Forschungszentrum Juelich) were placed in the middle of slabs to record root zone temperature, six in the cooling group and four in the control group. After the appearance of the 2nd inflorescences on 3rd January (84 DAS) and 6th June, 2018 (62 DAS), 10°C root temperature was applied until the final harvest.

2.2.3 Harvest and sample preparation

Harvest of fruits was done between 28th February to 6th April and 20th July to 29th August, 2018. After starting the treatment after the 2nd inflorescence, only the fruits from the 2nd to 5th cluster were harvested. Each cluster was separated into three parts, proximal, medium and distal, based on the distance to the stem. Each part was harvested when the fruits of this part became red and ripe. Total yield per plant was the combination of the mass of all the fruits from the 2nd to 5th cluster per plant. Marketable yield was the combination of the mass of all healthy, red and ripe fruits above 20g for 'Amoroso', while for 'Delioso', the minimum weight was 25g. Mean fruit weight was approximated by dividing total yield by total number of fruits per plant. After the fruits of the7th cluster were harvested, plant shoot length was also measured. Diameter at the base and internode between every two clusters were measured and averaged. After harvest, equatorial and longitudinal diameter (mm), and fresh weight (g) were performed on the fruits.

Two randomly selected red and ripe fruits from each part of two clusters (the 2nd and

3rd) were for further biochemical analysis. Two fruits were quartered and the seeds and locular tissue were removed. Two quarters from each fruit were pooled and quickly frozen in liquid nitrogen. The other two diagonal quarters were also pooled and were dried at 65°C for 48 hrs until constant weight. The frozen samples were ground in a kitchen coffee bean grinder (Clatronic International GmbH, Germany) with liquid nitrogen, and then stored at -80°C. The dried samples were ground in a mixer mill (MM400, Retsch, Germany).

2.2.4 Sugar quantification

Sugar concentrations were determined by enzymatic analysis according to Viola & Davies (1992) with minor adjustments. To 50 mg of frozen samples 400 µl 80% (v/v) ethanol was added, incubated at 80°C for 15 min and centrifuged for 3 min. Supernatant was saved and 400 µl 50% (v/v) ethanol was added to the pellet and re-extracted in the same method. This was repeated again three times using 200 µl 80% (v/v) ethanol. All supernatants were pooled together and ethanol was added to give 2 ml.

To determine the sugar concentrations, 20 µl aliquots were pipetted into 96 well plates and mixed with 160 µl enzyme mixture containing activated 1.12 units glucose-6-phosphate dehydrogenase (Roche diagnostics, Switzerland), 0.25 µmol NADP (Roche diagnostics, Switzerland), 0.5 µmol ATP (Merck, Germany), 0.75 µmol Mg^{2+} and measured at 340 mm by microplate reader (Synergy™ 2 Multi-Mode, BioTek, USA) at room temperature. When the extinction curve reached the plateau, 3.6 unit hexokinase (Roche diagnostics, Switzerland) was added. The same step was repeated until 1 unit phosphoglucose-isomerase (Roche diagnostics, Switzerland) and 20 unit invertase (Roche diagnostics, Switzerland) was successively added. Quantification of glucose, fructose and sucrose were determined photometrically by calculating absorbance increase in each stage, which was proportional to the sugar content in each well. Each sample was extracted and analyzed in duplicate. Extract

concentrations were converted into mg/g fresh weight and averaged.

2.2.5 Carotenoids determination

Carotenoids were consecutively extracted by acetone and ethyl acetate and analyzed by high-performance liquid chromatography (HPLC). Approximately 25 mg well-ground samples were weighed in 1.5 ml Eppendorf tubes and mixed with 1200 µl pre-cooled acetone (VWR, USA). After centrifuging at 13200 rpm for 15 min, the supernatant was carefully filtered through a 0.2 µm syringe filters (Sigma Aldrich, USA). The filtered solvent was divided into two 1.5 ml Eppendorf tubes to which 500µl water and 300 µl ethyl acetate were added. The mixture was then vortexed for 10 sec and centrifuged for 15 min at 13200rpm. Two ethyl acetate phases were combined and transferred to the amber vials for HPLC analysis.

Carotenoids were quantified by HPLC-PDA. Analyses were carried out on an Agilent 1220 Infinity II system (binary pump, autosampler, column oven) coupled to an Agilent photodiode array (PDA) detector (Agilent Technologies, USA). 20 µl standard and sample extract, respectively, was injected in the HPLC system. The carotenoids were separated on a ProntoSil 200-3-C30 column (250 x 4.6 mm, 3-µm particle size) and a corresponding guard column (10 x 4.0 mm, 3 µm) from Bischoff (Leonberg, Germany). The mobile phase consisted of methanol/water (99.3:0.7, v/v) containing 1 mM ammonium acetate (A) and tert-butyl methyl ether (B). Samples were separated at room temperature and a flow rate of 500 µl/min using a gradient program as follows: 85% A, linear gradient to 70% A over 12 min, isocratic at 70% A for 6 min, linear gradient to 15% A over 5 min and isocratic at 15% A for 5 min. Then the system was returned to its initial condition (85% A) within 5 min, and was equilibrated for 5 min before the next run was started (total run time: 55 min). The PDA detector was operated at wavelengths of 475 nm and 450 nm for lycopene and β-carotene,

respectively. Quantification was done by external calibration with corresponding standards (DHI, Denmark). The concentrations were converted into µg/g fresh weight and averaged.

2.2.6 Analysis of organic acid

Due to the similarity of structure and characteristics, three organic acids: citric acid, malic acid and ascorbic acid (Vitamin C) were extracted and analyzed simultaneously by Liquid Chromatography-Mass Spectrometry (LC-MS). Considering the matrix effect and high susceptibility of ascorbic acid to degrade, internal standard calibration $^{13}C_4$ malic acid was chosen. $^{13}C_4$ malic acid (Sigma-Aldrich, USA) was considered due to the similarity of chemical structure with other organic acids. 1200 µl extraction solvent containing 50 mg/l 1,4 dithiothreitol, and 50 mM ammonium acetate was added to approximately 20 mg sample along with internal standard ^{13}C malic acid to determine extraction efficiency. The sample was then homogenized at 4°C for 15 min and centrifuged at 4°C for another 15 min at 13200 rpm. The supernatant was then filtered through a 0.2 µm filter and diluted to the appropriate concentrations.

Quantification of organic acids was carried out using a Waters ACQUITY® UHPLC system (binary pump, autosampler) coupled to a Waters Xevo TQ-S® triple-quadrupole mass spectrometer (Waters Technologies Corp., MA, USA). Separation of three organic acids were achieved on a Nucleodur C18 Gravity-SB column (150 x 3 mm, 3 µm; Macherey-Nagel, Germany). The column was equipped with a precolumn (Macherey -Nagel, Germany). The mobile phase were water (A) and acetonitrile (B) each containing 0.1% formic acid, at a flow rate of 0.6 ml/min. The gradient program was as follows: 100% A isocratic for 4 min, to 97.5% A within 0.1 min, 97.5% A isocratic for 3.2 min, back to 100% A within 0.2 min and holding for 2.5 min. The electrospray ionization (ESI) interface of the mass spectrometer was

driven in the negative mode. The capillary voltage was set to 2.5 kV. The desolvation temperature and source temperature was 600°C and 150°C, respectively. The desolvation gas flow was set to 1000 l/h and the cone gas flow at 150 l/h using nitrogen in both cases. Mass spectrometric detection in the MRM mode was applied for quantification of the organic acids (Table 2.1). Nitrogen was used as the collision gas at a flow of 0.15 ml/min.

The concentration of each organic acid was determined by internal calibration using standard solutions composed of pure standard compounds (Sigma-Aldrich, USA). Each sample was extracted and analyzed in duplicate. Extract concentrations were converted into mg/g fresh weight and averaged.

Table 2.1 MRM parameter for organic acids. The 1st row of malic, ascorbic and citric acid is the quantifier mass transition and the 2nd row the qualifier mass transition used for quantification and compound confirmation, respectively.

Compound	Precursor ion [m/z]	Product ion [m/z]	Cone voltage [V]	Collision energy [V]
Malic acid	132.9	71	22	16
	132.9	114.95	22	12
13C4-malic acid	137	119	28	10
Ascorbic acid	175.02	87	24	16
	175.02	115	24	12
Dehydroascorbic acid	173	71	24	16
Citric acid	191.02	87	32	16
	191.02	111	32	12

2.2.7 Determination of carbon, nitrogen, sulfur and other elements

The ground dried sample was mixed with HNO_3, H_2O_2 and HF, integrated by microwave. Then ICP-OES (Inductively Coupled Plasma with Optical Emission Spectroscopy, Elan 6000, Perkin Elmer, Sciex; Agilent 7500ce, Planitz) was adopted to analyze P, K, Ca, Mg and Fe in the diluted sample solution. C and S concentrations of the

sample were determined by infrared absorption (Leco CS 600) based on the amount of CO_2 and SO_2 after conversion in flowing oxygen by radiofrequency heating. For the analysis of N, the samples were heated in flowing helium gas in a graphite crucible by means of resistance heating (Leco TCH 600).

2.2.8 Statistical analysis

All statistical analyses were performed by R studio (version 1.2.1335). Each plant was regarded as one biological replicate, the data from the 2nd and 3rd clusters and three positions within one cluster were averaged. Quality analysis data from each season and each cultivar was subjected to student's t-test was used. Experimental results were expressed as the means ± standard deviation.

2.3 Results and discussion

2.3.1 Greenhouse microenvironment parameters

In the winter of 2017-2018, the daily PPFD (Photosynthetic Photon Flux Density) and maximum air temperature inside the greenhouse ranged from 21.49-97.08 μmol/m^{2}s and from 19.84-29.17°C respectively (Figure 2.1). The values were higher in the summer of 2018, indicating 53.21 -125.75 μmol/m^{2}s and 23.49-39.01°C. Since the climate sensor was installed in the middle of the greenhouse, the PPFD values were below the actual values at plant level. The optimal range for tomato growth is 20-30 mol/m^{2}d (Jones Jr, 2007), and the requirements of plants were met with supplementary lighting and extended length.

In summer, the number of days exceeding daily maximum air temperature 30°C was 70, which covered half of the growth period and were mostly during fruit development. The

optimal daily temperature for fruit production is 19-20°C (Van Ploeg &Heuvelink, 2005). Air temperature above 32°C during the day caused reduction of pollen formation and viability (Adams et al., 2001; Domínguez et al., 2005). From this it could be assumed that plant growth was affected by extreme high air temperature during summer in our experiment. Root temperature in our control group was also higher in summer (19.78-26.47°C) than in winter (16.91-23.87°C).

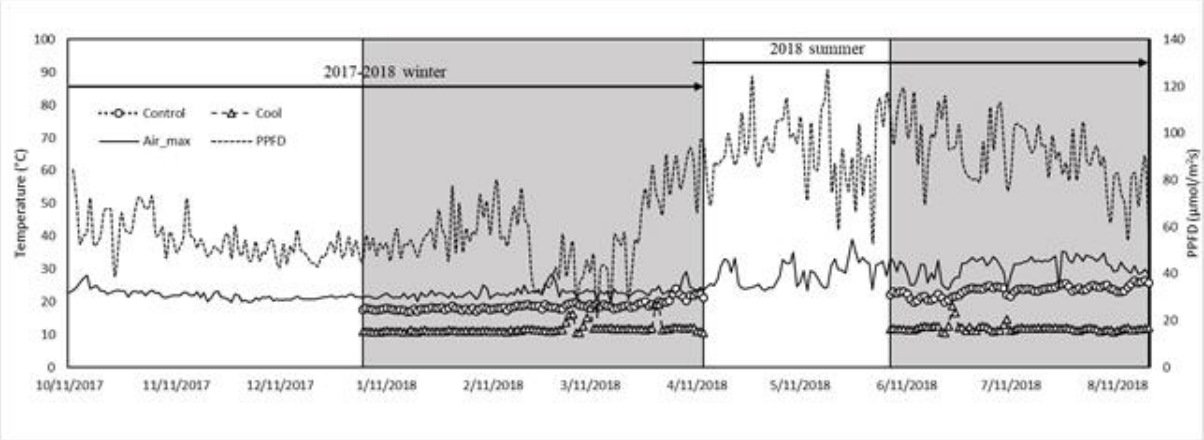

Figure 2.1 Moving average of daily maximum air temperature (Air_max), photosynthetic photon flux density (PPFD) recorded inside the greenhouse from 11 October 2017 to 20 August 2018.The two seasons were indicated by arrows at the top of the graph. Root temperature treatment in the two seasons were indicated as grey and root temperature from control group (open circles) and cooling group (open triangles) were recorded in the middle of Rockwool from 1 January to 6 April and 6 June to 20 August, 2018.

2.3.2 Influence of root cooling on plant growth and fruit yield

As indicated in Table 2.2, total yield and marketable yield per plant (the 2nd to 5th cluster) in both cultivars were reduced significantly at 10°C root temperature in the winter of 2017, but number, size and fresh weight of single fruit were not reduced significantly by root cooling. Besides, 'Delioso' showed greater magnitudes of reduction by 17.9% in total yield and 20.9% in marketable yield. This is in contrast to an experiment by Fujimura et al.,

(2012), where not only the total yield of tomato was reduced at 12°C root temperature, but also fruit size and fruit number, regardless of the season. Many other studies further confirm the assumption that low root temperature leads to a reduced shoot growth and is mainly attributed to water stress (Wan et al., 2001; Murai-Hatano et al., 2008). Reduction in water uptake leads to the stomata closure in order to maintain positive turgor pressure within the plant. The resultant CO_2 uptake and net photosynthetic rate became reduced, with eventual restriction of carbon production (Wan et al., 1999). By contrast, Fujimura et al. (2012) observed that the photosynthesis rate and stomatal conductance of tomato plants were not significantly affected by root chilling at 12°C and suggested that the tomato plants showed acclimation within one week. Yan et al., (2013) or Zhang et al., (2012) suggested that loss of root cell viability and membrane lipid peroxidation inhibited plant growth. Unfortunately, root growth, respiration and biomass were not measured in our studies due to the structure of rockwool. In our studies, the reduction of water content in the fruits of "Delioso" in winter (Table 2.2) confirmed that the plants suffered from water stress induced by root cooling. But water stress is not the only reason, otherwise, the yield should be reduced even more in summer. Other studies attributed restriction of shoot growth under low root temperature to the imbalance between growth promoters and inhibitors, such as cytokines, abscisic acid and gibberellins (Bugbee & White, 1984; Ntatsi et al., 2013), which are primarily synthesized in root apical meristems (Taiz & Zeiger, 2007). Additionally, secondary metabolites, induced by abiotic and biotic stress, consumed more energy. Because carbon distribution was diverted to the production of secondary metabolites, and this resulted in the reduction of plant growth and development as well as the yield (Hofmann & Jahufer, 2011; Huot et al., 2014).

Table 2.2 Effects of root cooling on yields, fruit and shoot growth parameters of cocktail tomato plants in the two seasons. Significant differences ($p < 0.05$) are indicated in bold.

			Fruit								Shoot	
			Total Yield (g) /Plant	Marketable Yield (g)/Plant	Marketable /Total (%)	Mean weight (g)	Equatorial Diameter (mm)	Longitudinal Diameter (mm)	Number of Fruits / Plant	Water content (%)	Height (cm)	Diameter (mm)
2017-2018	'Amoroso'	Control	1908.76± 96.6	1898.7 ± 89.9	99.5	35.7 ± 0.9	40.6 ± 0.7	33.1 ± 0.7	53.3 ± 3.6	94.68 ± 0.54	197.2 ± 16.1	16.2 ± 1.4
Winter		Cool	1762.1 ± 109.1	1748.6 ± 116.7	99.2	32.3 ± 3.3	39.4 ± 1.2	32.4 ± 0.8	53.0 ± 2.2	94.34 ± 0.44	190.0 ± 8.0	15.0 ± 1.0
	p-value		**0.047**	**0.049**		0.052	0.063	0.103	0.850	0.270	0.357	0.145
	'Delioso'	Control	2117.2 ± 273.0	2095.9 ± 266.6	97.3	39.2 ± 2.2	41.2 ± 1.0	32.1 ± 0.8	53.5 ± 7.4	94.91 ± 0.07	215.8 ± 20.6	14.1 ± 0.9
		Cool	1738.5 ± 123.6	1657.1 ± 179.8	95.3	36.0 ± 5.4	39.8 ± 2.2	31.0 ± 1.8	49.0 ± 6.2	93.98 ± 0.57	215.4 ± 10.3	13.9 ± 1.1
	p-value		**0.018**	**0.011**		0.264	0.233	0.238	0.300	**0.010**	0.966	0.721
2018	'Amoroso'	Control	1310.8 ± 157.9	1168.2 ± 164.6	89.1	35.0 ± 3.1	37.6 ± 2.3	32.3 ± 2.3	37.7 ± 5.0	94.10 ± 0.35	112.9 ± 8.2	20.1 ± 1.0
Summer		Cool	1489.1 ± 171.3	1299.2 ± 219.8	87.2	34.3 ± 2.7	37.9 ± 0.9	32.8 ± 0.8	43.3 ± 3.0	94.25 ± 0.68	125.6 ± 7.4	18.9 ± 1.6
	p-value		0.135	0.272		0.721	0.823	0.643	**0.018**	0.646	**0.019**	0.186
	'Delioso'	Control	1682.0 ± 372.9	1652.4 ± 405.3	98.2	41.6 ± 6.2	41.5 ± 2.6	34.1 ± 2.1	40.0 ± 4.1	93.31 ± 0.71	130.2 ± 7.9	18.8 ± 1.1
		Cool	1636.4 ± 392.7	1527.4 ± 488.1	93.3	41.7 ± 10.0	41.7 ± 3.9	34.8 ± 2.9	39.3 ± 2.7	93.93 ± 0.79	134.2 ± 6.0	16.8 ± 1.3
	p-value		0.839	0.640		0.991	0.920	0.662	0.748	0.188	0.341	**0.013**

In summer, the yield was not affected by root cooling, and 'Amoroso' even showed an increase in total and marketable yield with more fruits (Table 2.2), though not significantly. Most of the daily maximum air temperatures during the fruit development stage were above 30°C in the summer of 2018 (Figure 2.1). These results are similar to these reported by Adams et al. (2001) and Domínguez et al. (2005), which mentioned that air temperature above 32°C caused a reduction of pollen formation and viability. Thus, fruits of the 4th and 5th cluster only developed one fruit on some of the plants, leading to a reduction of overall yield and number of fruits in the control group (Data not shown). Again, in the daytime, the root temperature in the control group was around 27°C, with maximum up to 30°C (Figure 2.1). Díaz-Pérez et al. (2007) mentioned that high root temperature above 27°C resulted in reduction of plant growth and fruit yield. The increase in total and marketable yield under low root temperature (Table 2.2) indicated that root cooling to some extent relieved heat stress from aboveground for 'Amoroso' in our experiment. Similarly, fruit number and weight of cucumber were increased with root-zone cooling at high ambient temperature (Moon et al., 2006). Furthermore, Mohammud et al. (2016) as well as Kii & Araki (2016) also proved that water chilling through the root zone of tomato during summer could improve the fruit yield by increasing the fruit number and average fruit weight. The response of root cooling in our two seasons, experiments and other environmental factors, such as light and air temperature interacting with root temperature influenced the final yield. These results are in accordance with other studies on the interaction of root-zone and air temperature under given lighting conditions as recently reviewed by Kawasaki & Yoneda (2019).

In winter, both cultivars did not have significant differences in the shoot length and average diameter as a result of root cooling, as shown in Table 2.1. The effect of root cooling was also dependent on the period of treatment. Wan et al. (1999) observed a sharp reduction

of water uptake and stomatal conductance at 24 hrs after 5°C root temperature treatment. Fennell & Markhart (1998) reported acclimation of stomatal conductance in spinach after three days' 5°C root temperature treatment. In our studies, growth of shoot might also show acclimation after two-three months' treatments and hence, no statistically significant differences were observed. However, in 2018 summer, plant growth presented greater changes with longer and slender shoot as a result of treatment. Because in summer, the root cooling treatment started early (62 DAS) than in winter (84 DAS), it was assumed that the differences were caused mainly in the early stages of treatment, during which the shoot height was below 30 cm.

2.3.3 Influence of root cooling on bioactive compounds

Glucose and fructose are the major sugars present in both walls and locular of mature tomato fruits, accounting for nearly half of the total dry matter of tomato fruits, hence influencing tomato fruit quality largely (Kader, 2008). The levels of glucose and fructose in the control group ranged from 17.6 to 20.7 mg/g FW and 13.9-18.9 mg/g FW. These values were consistent with other studies, reporting ranges from 6.8 to 17.9 g/kg FW in tomatoes of different sizes (Atkinson et al., 2011; Figàs et al., 2015). The levels of sucrose in control samples (ranging from 0.25-1.92 mg/g FW) were higher compared with previous reports where sucrose is less than 0.05% of fresh weight (Lu et al., 2010). The low levels of sucrose could be due to degradation of sucrose to glucose after transportation from the phloem (Koch, 2004). In winter, glucose and fructose levels were strongly influenced by root temperature (Table 2.3). The treatment showed higher values in glucose and fructose with increments of 7.8% and 7.4% respectively 'Amoroso'. The rise of glucose (10.3%) and fructose (13.3%) concentrations in 'Delioso' at low root temperature were even stronger. However, this effect was not significant for sucrose concentration in both cultivars. Similarly, in the summer of

2018, glucose levels increased by 6.9% and 7.7% in 'Amoroso' and 'Delioso', respectively. The levels of fructose showed an increase by root cooling as well, however, not significant (Table 2.3). Soluble sugars are important signaling molecules to regulate carbohydrates metabolism when plants are exposed to abiotic stress (Patrick et al., 2013). When exposed to low-temperature stress, soluble sugars can act as osmoprotectants to keep a turgor pressure, nutrients for plants to survive the stress, interactor with lipid layer protecting cellular membranes as, hormone-like primary messengers as ABA, antioxidant-like scavengers (Zhang et al., 2007; Ma et al., 2009; Sami et al., 2016). Another explanation is that, the reduction in sink strength of roots when exposed to suboptimal temperature without down-regulation of net photosynthesis (Ntatsi et al., 2014a). Water stress caused by low root temperature could also explain the increase in sugar concentrations which accompany a reduction in water. However, Fujimura et al. (2012) denied this cause, and attributed it to an excess of photoassimilates led by the imbalance of sink and source capacity to suboptimal temperature.

Table 2.3 Effects of root cooling on sugar, organic acid and carotenoids concentrations of cocktail tomato fruits. Significant differences ($p<0.05$) are indicated in bold.

			Sugar (mg/g FW)			Organic acid (mg/g FW)			Carotenoids (µg/g FW)	
			Glucose	Fructose	Sucrose	Malic acid	Citric acid	Ascorbic acid	Lycopene	β-carotene
2017-2018 winter	'Amoroso'	Control	18.91 ± 0.77	15.33 ± 0.79	1.32 ± 0.34	1.08 ± 0.11	5 ± 0.62	0.23 ± 0.01	72.48 ± 5.79	7.51 ± 0.36
		Cool	20.38 ± 0.95	16.47 ± 0.68	1.53 ± 0.21	1.09 ± 0.1	5.15 ± 0.31	0.22 ± 0.02	82.25 ± 10.67	8.28 ± 0.71
		p-value	**0.015**	**0.024**	0.228	0.820	0.611	0.734	0.086	**0.047**
	'Delioso'	Control	19.08 ± 1.01	15.63 ± 1.23	0.91 ± 0.29	1.04 ± 0.12	4.75 ± 0.65	0.19 ± 0.01	85.08 ± 5.85	7.60 ± 0.62
		Cool	21.04 ± 1.17	17.72 ± 0.68	1.28 ± 0.12	0.99 ± 0.20	4.89 ± 0.61	0.23 ± 0.01	102.5 ± 5.63	8.43 ± 0.54
		p-value	**0.045**	**0.033**	0.07	0.627	0.710	**0.001**	**0.002**	0.055
2018 summer	'Amoroso'	Control	19.44 ± 1.03	17.49 ± 0.90	0.59 ± 0.19	0.29 ± 0.04	4.22 ± 0.28	0.23 ± 0.01	79.55 ± 6.90	9.76 ± 0.64
		Cool	20.78 ± 0.94	17.99 ± 0.76	0.71 ± 0.11	0.27 ± 0.06	4.28 ± 0.55	0.25 ± 0.02	72.52 ± 5.95	9.45 ± 0.63
		p-value	**0.041**	0.327	0.242	0.619	0.821	0.019	0.088	0.411
	'Delioso'	Control	18.41 ± 0.74	16.85 ± 0.66	0.99 ± 0.28	0.26 ± 0.02	3.60 ± 0.13	0.20 ± 0.01	79.72 ± 2.25	10.66 ± 0.74
		Cool	19.82 ± 0.70	17.70 ± 0.64	1.24 ± 0.23	0.26 ± 0.04	3.91 ± 0.38	0.24 ± 0.02	93.17 ± 3.08	10.22 ± 1.23
		p-value	**0.015**	0.071	0.160	0.700	0.105	**0.003**	**<0.001**	0.525

Citric acid and malic acid are the major non-volatile organic acids, responsible for the sourness in tomato fruits (Marconi et al., 2007). And the total organic acids were generally positively related to total acidity (Anthon et al., 2011). In line with previous studies, the levels of citric acid in control samples (ranging from 3.4 to 6.0 mg/g FW) were 4 to 5 times higher than that of malic acid (0.22-1.21 mg/g FW) (Hernández et al., 2008; Anthon et al., 2011). In the two seasons and two cultivars, the concentration of citric acid and malic acid did not exhibit differences as a result of root cooling. These findings were similar to the results of Fujimura et al. (2012), the concentration of malic acid and citric acid were not influenced by root cooling regardless of the cultivar or the season. Shaw (1990) also confirmed that total acidity was less controlled by environmental factors than genetic traits. Both cultivars showed higher levels of malic acid (0.88-1.21 mg/g FW) and citric acid (4.17-6.00 mg/g FW) in winter, especially for the values of malic acid. In contrast, strawberries planted in summer contained higher levels of titratable acidity than in winter (Kader, 1991). Lobit et al. (2006) modeled the vacuolar malic acid concentration of peach fruit versus air temperature, and observed a reduction of about 50% with an increase in air temperature from 15 to 20°C. Wang & Camp (2000) also proved that the concentrations of organic acids in strawberry grown at high temperature were lower, probably due to higher respiration rate. Higher air temperature in 2018 summer might have led to lower organic acid concentrations in the fruits of our tomatoes.

In this study, two cultivars showed similar ascorbic acid concentration compared with other studies, which reported values ranging from 1.6 to 6.4 mg/g FW of cherry tomatoes harvested at different times of the year (Raffo et al., 2002). The ascorbic acid concentrations of the treated samples were 20-21% higher than that of the control (no root controlling) in 'Delioso' in winter and summer. On the other hand, ascorbic acid in ‘Amoroso’ only exhibited higher (8.7%) value in summer. As expected, vitamin C changed as a result of the cooling

treatment and the increment was mainly due to the protection mechanism from low temperature-induced oxidative stress (Hidaka et al., 2007). Nonetheless, both cultivars contained slightly higher concentrations in summer than those harvested in winter, which is in accordance with Massot et al. (2010) and Roselló et al. (2011). Seasonal variations in ascorbate levels have been attributed to gene expression of biosynthesis and recycling, regulated by the interaction of temperature and light during the season (Johkan et al., 2013; Massot et al., 2013).

Amongst the carotenoids, only lycopene and β-carotene were measured in our studies. Lycopene predominates 60-74% of the carotenoids and is responsible for the red color (Langi et al., 2018). The lycopene values reported in the present study (ranging from 71.3-108.9 μg/g FW) were similar to early studies (ranging from 15 to 160 μg/g FW) depending on different cultivar, ripeness, environmental factor, agricultural practices and post-harvest storage conditions (Martínez-Valverde et al., 2002; Gautier et al., 2005; Thompson et al., 2000; Zanfini et al., 2016). In both seasons, low root temperature caused 16.9-20.5% accumulation of lycopene in 'Delioso', but had no impact on 'Amoroso'. Fruit temperature plays an essential role in the synthesis of lycopene, below 12°C or above 30°C during the fruit stage, synthesis of lycopene was inhibited (Krumbein et al., 2006; Roselló et al., 2011). The concentration of lycopene has been reported to be lower in the summer months than other times due to high air temperatures and excessive sunlight (Toor et al., 2006; Rosales et al., 2006). Krumbein et al. (2006) observed that the optimal air temperature range for lycopene accumulation was 20-24°C. In winter, air temperature was in the optimal range, and the main stress for plants was from root cooling. Consequently, 'Delioso' behaved more sensitively to root cooling by increasing the concentration of lycopene. As mentioned earlier, daily maximum air temperature during fruit development in summer was above 30°C. Seasonal changes in lycopene levels have been attributed to both heat stress aboveground and

cold stress belowground. In summer, ‘Delioso’ increased concentration of lycopene under lower root temperature, probably root cooling partially alleviated the negative impacts of heat stress. However, the effects for 'Amoroso' were not obvious. Therefore, biosynthesis of lycopene was a result of the comprehensive effect of air temperature, root-zone temperature, solar irradiation and other climatic factors.

β-carotene, a precursor of vitamin A, is associated with the orange color in tomatoes (Langi et al., 2018). The levels of β-carotene in control samples (ranging from 7.0-11.5 μg/g FW) were lower than those reported by other authors in the range of 9.8-16.7 mg/kg FW (Gautier et al., 2008). In winter, the levels of β-carotene with root cooling was 10% higher than the control group in 'Amoroso', while no differences were observed in 'Delioso'. In summer, the levels of β-carotene did not show differences in both cultivars when exposed to root cooling, but with higher levels than winter. β-carotene concentration was positively related to light intensity, and less affected by high air temperature up to 38°C (Brandt et al., 2003). Therefore higher light intensity accounted for the increase of β-carotene levels in summer. Lycopene and β-carotene indicated different sensitivity and the response was dependent on the genotypes, which were consistent with Roselló et al. (2011) and Gautier et al. (2005).

Low air temperature was known to cause stress for plants, which lead to down-regulation of Calvin cycle and accumulation of reactive oxygen species (ROS), such as $^1O_2$, O_2^-, H_2O_2 and •OH (Wang et al., 2004; Ensminger et al., 2006). To counteract the deleterious oxidative damage of ROS, plants have to produce antioxidant enzymes and antioxidants (He et al., 2014), such as ascorbate and carotenoids, as defense systems. With the application of low air temperature, levels of sugars and antioxidants were increased in the spinach (Kato, 1995); concentration of vitamin C increased in strawberry (Wang & Camp, 2000). Likewise,

extreme root temperature stress also leads to the increased production of various metabolites in plants. In red romaine lettuce and red leaf lettuce, 10 °C root temperature accelerated the accumulation of anthocyanin, phenols and sugars than other temperatures (Sakamoto & Suzuki, 2015a; Islam et al., 2019). Cucumber seedlings had higher sugar concentrations at 12°C than 20°C root temperature (Yan et al., 2013). In two medicinal plants: *Catharanthus roseus* and *Nicotianna tabacum*, the biosynthesis and accumulation of alkaloid were increased by altering root temperature during 48h root temperature treatment (Malik et al., 2013). In agreement with previous studies, higher concentrations of glucose, fructose, ascorbic acid, lycopene and β-carotene were observed as a function of root cooling in our experiments. The response of cultivar to root temperature was different, depending on the sensitivity to cooling and other environmental factors.

2.3.4 Influence of root cooling on elements concentrations

Already Pollock & Eagles (1988) reported that the effect of low temperature on carbon fixation and translocation is relatively mild, which was true in our case (Table 2.4). Concentration of carbon did not vary significantly with treatment in both seasons, except for 'Amoroso' in the summer of 2018 with a reduction of 0.8%. It is commonly believed that carbon is accumulated in leaves in response to low root temperature. And this accumulation is attributed to reduced translocation rates and decreased sink demand by cold roots (Nagel et al., 2009; Poire et al., 2010). By contrast, Wilson (1988) and Hamblin et al. (1990) observed an increased fraction of carbon to root under low temperature for the maintenance of construction and respiration, however, these changes seem to depend on species and cultivars types. Carbon concentration in the fruit is the result of the balance between carbon partitioning and respiration. Carbon portioning between sink and source is also influenced by other environmental factors. The higher air temperature in summer complicated the carbon

accumulation in fruits, and may explain the inconsistent results of two seasons for 'Amoroso'.

The results indicated a large number of elements reduced concentration in 'Delioso' during winter, especially N, P, S, Fe with varying reductions of 12.1%, 15.7%, 13.3% and 15.4% respectively (Table 2.4). However, all the nutrients were not affected by root temperature in 'Amoroso' at the same time. In summer, both the levels of macro and micro elements in 'Amoroso' showed a general reduction as a function of root cooling, but only P levels decreased by 15.0% and Zn values decreased by 22.2% significantly. In response to root cooling, 'Delioso' showed statistical similarities in P and Zn concentrations by reduction of 9.1% and 13.7% respectively. But Mg values increased by 11.1% in 'Delioso'. Cooper (1973) described tomato as one representative species, in which the mineral concentrations increase with root temperature, achieve the highest at one point, and afterwards, concentrations decline. And this optimal point is around 25°C based on the different cultivars and light conditions (Tindall et al., 1990). Concentrations of ions in the fruits were influenced by root uptake, transport from root to shoot and dilution effect caused by growth as well (Quadir et al., 2011). Considering the reduction in fresh biomass of fruits in our studies, the dilution effects by growth could be excluded.

Table 2.4 Effects of root cooling on elements concentrations of cocktail tomato fruits in two seasons. Significant differences (p<0.05) are indicated in bold.

	2017-2018 winter						2018 summer					
	'Amoroso'			'Delioso'			'Amoroso'			'Delioso'		
	Control	Cool	*p*-value	Control	Cool	*p*-value	Control	Cool	*p*-value	Control	Cool	*p*-value
C (%)	44.16 ± 0.22	44.19 ± 0.25	0.858	43.5 ± 0.72	43.61 ± 0.51	0.768	45.81 ± 0.28	45.42 ± 0.13	**0.017**	45.63 ± 0.42	45.73 ± 0.45	0.696
N (%)	1.93 ± 0.21	1.85 ± 0.08	0.399	1.74 ± 0.12	1.53 ± 0.10	**0.011**	2.21 ± 0.11	1.97 ± 0.26	0.077	2.12 ± 0.18	2.03 ± 0.11	0.320
K (%)	4.36 ± 0.29	4.43 ± 0.10	0.582	4.68 ± 0.25	4.38 ± 0.54	0.254	3.01 ± 0.24	2.75 ± 0.26	0.107	3.29 ± 0.15	3.32 ± 0.27	0.797
P (%)	0.50 ± 0.03	0.48 ± 0.02	0.152	0.51 ± 0.03	0.43 ± 0.06	**0.030**	0.40 ± 0.03	0.34 ± 0.04	**0.028**	0.44 ± 0.03	0.40 ± 0.02	**0.019**
S (%)	0.15 ± 0.01	0.15 ± 0.01	0.284	0.15 ± 0.00	0.13 ± 0.01	**0.004**	0.19 ± 0.01	0.18 ± 0.02	0.839	0.20 ± 0.01	0.20 ± 0.02	0.749
Ca (%)	0.07 ± 0.01	0.06 ± 0.00	0.276	0.06 ± 0.01	0.05 ± 0.00	0.073	0.08 ± 0.01	0.09 ± 0.01	0.118	0.10 ± 0.01	0.09 ± 0.01	0.641
Mg (%)	0.10 ± 0.01	0.09 ± 0.01	0.585	0.10 ± 0.02	0.09 ± 0.01	0.265	0.08 ± 0.01	0.08 ± 0.01	0.866	0.09 ± 0.01	0.10 ± 0.01	**0.027**
Zn (mg/kg)	19.43 ± 3.18	21.16 ± 2.84	0.343	22.05 ± 4.83	18.88 ± 2.51	0.194	21.16 ± 2.84	16.47 ± 2.75	**0.016**	21.68 ± 2.39	18.71 ± 0.65	**0.027**
Fe (mg/kg)	53.10 ± 5.00	50.84 ± 8.24	0.582	53.4 ± 7.02	45.2 ± 4.60	**0.042**	------	-----		-----	-----	
Na (mg/kg)	148.16 ± 14.09	152.39 ± 9.86	0.562	179.06 ± 12.47	183.19 ± 19.43	0.672	82.18 ± 11.43	92.89 ± 15.61	0.208	91.79 ± 24.58	97.53 ± 40.74	0.775
Mn (mg/kg)	5.95 ± 0.50	5.47 ± 0.55	0.144	5.66 ± 1.52	4.91 ± 0.63	0.303	6.49 ± 1.50	4.93 ± 0.65	0.052	6.28 ± 0.67	5.40 ± 0.93	0.095

---- Iron concentrations in 2018 summer was below the detection limit.

In short-term, uptake of ions were severely affected by suboptimal root temperature has been further proved in other species: grapevine (Clarke, et al., 2015), African snake tomato (Adebooye et al., 2010), spring barley (Füllner, 2007), rice (Jia et al., 2019), lettuce (Tan et al., 2002). Altered root morphology under low root temperature was one reason (Nagel, et al., 2009; He et al., 2009). The mobility of cellular membrane phospholipids was lost induced by low root temperature below the phase transition point (Badea & Basu, 2009). Thus, ions carrier proteins and enzymes function were hampered (Ahn et al., 2000; Feng et al., 2011). Another factor was related to limited energy available for ion uptake caused by reduced root respiration with root chilling (Sakamoto & Suzuki, 2015), which also, in turn, inhibited ion carriers' function. Reduced root hydraulic conductivity, which regulates water and nutrient uptake, was another reason suggested by George et al. (2002) or Lee et al., (2004). However, tomato plants of our studies were exposed to low root temperature for extended periods. White et al. (1987) & Engels et al. (1992) indicated that roots increased the capacity of ion uptake after long exposure to low root temperature. This acclimation is currently attributed to the adaptation of previously mentioned alterations caused by low root temperature, e.g. increased ion transporters in the plasma membrane, insensitive flux of ions into the xylem (reviewed by Janská et al., 2010), enhanced hydraulic conductivity (Ahamed et al., 2012) and increased shoot demand with growth. But this adaption to low root temperature was not detected if the shoot base temperature was within the cooling zone (Engels et al., 1992). In our setup, the isolation matt covered and wrapped the shoot base, and consequently the base temperature was reduced as well. The uptake of N, P, K, S, Fe, Zn did not demonstrate acclimation. On the contrary, the increased level of Mg in 'Delioso' could be explained by the long-term adaptation to root cooling.

The concentrations of nutrients in the fruits were also dependent on the translocation of minerals from root to shoot through xylem. The translocation rate was determined by a

complex process: uptake rate of nutrients, retention of nutrients in root or for root growth, and transpiration rate (Quadir et al., 2011). Ca, P, K in the leaf of snake gourd (*Trichosanthes cucumerina* L.) at sup-optimal root temperature preferred to retain in the root than relocation to the shoot. Adebooye et al. (2010) found that the partitioning of Fe and Mn was not influenced by root temperature. In the present study, the reduction of N, P, S, Fe, Zn concentrations of fruits could also be explained by the lower translocation rate to fruits at low root temperature.

Other hypotheses also exist for the altered concentrations of minerals in shoots as a function of low root temperatures. Iron levels were observed to be higher in the leaf of spinach and rice at low root temperature, and the increment was attributed to the enhanced biosynthesis of isozymes such as Fe-SOD, Zn-SOD and Mn-SOD (Kitano et al., 2008; Chadirin et al., 2011). In our tomato fruits, the concentrations of Fe, Zn, Mn were either decreased or unaffected. It was assumed that the increased amount caused by the isozymes, may not compensate for the reduced amount by uptake or translocation.

Chapter 3

Effects of root temperature on the plant growth and food quality of Chinese broccoli (*Brassica oleracea* var. *alboglabra* Bailey)

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3.1 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 (Chapin, 1974). In hydroponic systems, root temperature is generally different from ambient temperature (Sun et al., 2016) 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 (Setter and Greenway, 1988; Díaz-Pérez, 2009), photosynthesis and transpiration (Adebooye et al., 2010; Aidoo et al., 2019). 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 (Yan et al., 2013; Sakamoto and Suzuki, 2015a & b), while still other works found the quality of strawberries unaffected by root temperature (Sakamoto et al., 2016).

Vegetables in the Brassica genus are consumed around the world and are important for human and animal health (Rosa et al., 2001). Chinese broccoli belongs to the same species *Brassica oleracea* (common broccoli) but in the variety group *alboglabra* (Chen et al., 2009). 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 (Qian et al., 2016). Its abundance in health-promoting antioxidants

and essential minerals (Deng et al., 2017) resulted in greater Chinese broccoli consumption in Europe and America in recent years (Sun et al., 2012 a & b).

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 (Fuentes et al., 2015). The pungent flavor and bitter taste of Brassica vegetables are associated with the breakdown products of glucosinolates isothiocyanates (Wieczorek et al., 2018). Sugar levels, together with glucosinolates, also influence the flavor and acceptance by the consumer (Rosa et al., 2001). 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 (Kawashima & Valente Soares, 2003). Air temperature has been reported to influence the levels of glucosinolates and sugars in curly kale and turnips (Steindal et al., 2015; Justen and Fritz, 2013). Previous studies have also been conducted into the effects of rooting environment temperature on certain Brassica vegetables (Díaz-Pérez, 2009; Cumbus & Nye, 1982; Cumbus & Nye, 1985; Macduff et al., 1987; Kleier et al., 1998). 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 (Díaz-Pérez, 2009). 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) of Chinese broccoli without reducing the yield.

3.2 Materials and Methods

3.2.1 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 (Qian et al. 2016) 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 $CaNO_3$, 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). 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 $CaNO_3$, 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) 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.

3.2.2 Root temperature setup

Two experiments were carried out under greenhouse conditions (Forschungszentrum Jülich, Germany) from 2017 to 2018 as shown in Table 3.1. 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 3.1) 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 3.1 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

3.2.3 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) (Sun et al., 2011). Each plant was a replicate. After harvest, shoot height was measured from cotyledon scar to base of petiole on the 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 (Sun et al., 2011). The edible parts were separated into stems 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.

3.2.4 Elemental analysis

Ground dried samples were digested in HNO_3, H_2O_2 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 (2019).

3.2.5 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 and Davies (1992) 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^{2+}, 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.

3.2.6 Glucosinolates analysis

The extraction of intact glucosinolates from stems and leaves was performed according to the method of Volden et al. (2009) and Doheny-Adams et al. (2017) 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 (degaser, 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^{-1}. 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^{-1}, respectively. The nebulizer pressure was set to 60 psi. Mass spectrometric detection in the MRM mode was applied for the quantification of glucosinolates (Table 3.2).

The concentration of each glucosinolate was determined by external calibration using a standard solution composed of pure standards compounds (Phytoplan 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).

Table 3.2 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]**
Aliphatic	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
Indolic	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

3.2.7 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 preference testings between treatments and determine the probability of statistical difference.

3.3 Results

3.3.1 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 3.1). 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.

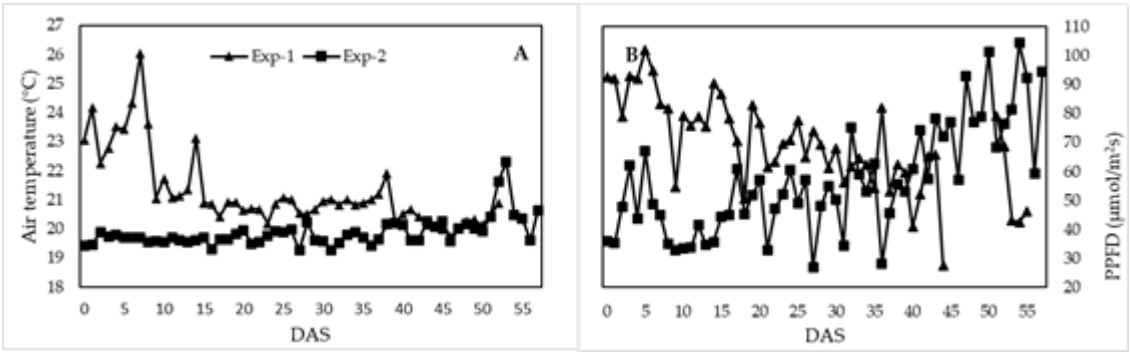

Figure 3.1 Daily average air temperature (**A**) and PPFD (photosynthetic photon flux density) (**B**) recorded during Exp-1 and Exp-2 to test the influence of rooting temperature on Chinese broccoli growth and quality in a glasshouse. DAS: days after sowing.

3.3.2 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 3.3), 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 3.3) 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 3.2). The negative effect on shoot biomass was dependent on the treatment duration (Figure 3.2) and the final yield was 26% lower at 10 °C than 20 °C (Table 3.3). 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 3.2). 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 3.3). 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 3.3 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**	***p*-value**	**10 °C**	**20 °C**	***p*-value**
Shoot	**Yield (g)**	**153.5 ± 21.2**	**154.7 ± 22.2**	**0.878**	**137.2 ± 9.0**	**172.9 ± 32.2**	**0.041**
	FW (g)	**170.4 ± 21.6**	**175.5 ± 21.6**	**0.511**	**153.4 ± 13.7**	**218.0 ± 24.7**	**<0.001**
	DW (g)	**14.83 ± 2.16**	**15.38 ± 2.37**	**0.499**	**14.52 ± 0.99**	**17.40 ± 2.09**	**0.018**
	Height (cm)	**35.30 ± 5.84**	**35.77 ± 4.53**	**0.802**	**34.35 ± 3.82**	**41.55 ± 4.35**	**0.013**
	Shoot ratio (%)	**92.94 ± 0.61**	**94.37 ± 0.86**	**<0.001**	**93.47 ± 0.34**	**95.37 ± 1.22**	**0.011**
Root	**FW (g)**	**15.32 ± 2.37**	**11.28 ± 1.63**	**<0.001**	**19.58 ± 1.23**	**16.67 ± 2.83**	**0.055**
	DW (g)	**1.12 ± 0.16**	**0.91 ± 0.15**	**<0.001**	**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**	**0.029**

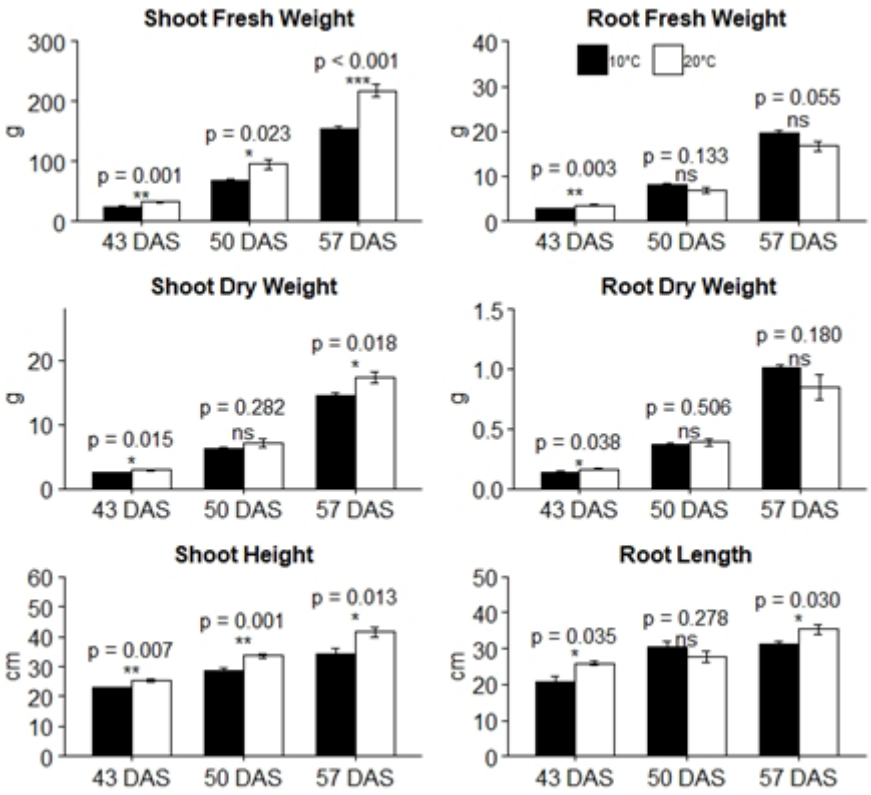

Figure 3.2 Effects of root temperature (10 and 20°C) on shoot and root fresh weight, shoot and root dry weight, shoot height and root length of Chinese broccoli at three harvest dates (43 DAS, 50 DAS and 57 DAS) during Exp-2. Levels of significance are represented by ns not significant, $*$ $p < 0.05$, $**$ $p < 0.01$ and $***$ $p < 0.001$. DAS: Days after sowing.

3.3.3 Effects of root remperature 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 3.4). 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 3.4. 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 stems 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 age. 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 significant with crop age. However, the nitrogen level of the roots was higher at 10 °C throughout the experiment. Phosphorus concentrations were lower in 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 and Mg in 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 3.4 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	*p*-value	10 °C	20 °C	*p*-value	10 °C	20 °C	*p*-value	10 °C	20 °C	*p*-value
Leaves	C	38.75 ± 0.58	38.63 ± 0.63	0.583	39.89 ± 0.47	36.55 ± 0.62	**<0.001**	37.91 ± 0.89	34.86 ± 0.56	**<0.001**	38.28 ± 0.84	36.81 ± 0.99	**0.021**
	N	6.35 ± 0.15	6.39 ± 0.11	0.361	5.32 ± 0.09	6.34 ± 0.12	**<0.001**	5.06 ± 0.21	6.19 ± 0.12	**<0.001**	5.4 ± 0.41	5.93 ± 0.29	**0.028**
	P	0.63 ± 0.07	0.62 ± 0.04	0.887	0.48 ± 0.04	0.66 ± 0.06	**0.001**	0.52 ± 0.07	0.62 ± 0.05	**0.032**	0.69 ± 0.04	0.78 ± 0.06	**0.011**
	K	2.89 ± 0.29	2.85 ± 0.27	0.682	5.01 ± 0.45	6.14 ± 0.21	**0.003**	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	**<0.001**	4.41 ± 0.81	5.63 ± 0.74	**0.038**	4.35 ± 1.12	5.05 ± 0.95	0.270
	Mg	0.55 ± 0.06	0.46 ± 0.04	**<0.001**	0.62 ± 0.07	0.91 ± 0.06	**<0.001**	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	**0.001**	39.7 ± 0.95	36.86 ± 0.87	**0.001**	38.96 ± 0.69	37.2 ± 0.88	**0.004**
	N	4.53 ± 0.35	4.35 ± 0.18	0.094	2.94 ± 0.22	4.01 ± 0.18	**<0.001**	3.88 ± 0.17	4.32 ± 0.23	**0.010**	3.69 ± 0.16	3.94 ± 0.14	**0.016**
	P	0.49 ± 0.04	0.51 ± 0.05	0.293	0.38 ± 0.06	0.53 ± 0.05	**0.003**	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	**0.003**	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	**0.014**	1.17 ± 0.17	1.48 ± 0.22	**0.020**
	Mg	0.29 ± 0.03	0.26 ± 0.03	**0.048**	0.39 ± 0.07	0.40 ± 0.04	0.773	0.45 ± 0.06	0.55 ± 0.04	**0.014**	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	**<0.001**	5.4 ± 0.44	4.46 ± 0.26	**0.006**	5.17 ± 0.15	3.82 ± 0.55	**0.004**	4.44 ± 0.37	3.82 ± 0.54	**0.045**
	P	0.95 ± 0.14	0.96 ± 0.11	0.69	0.69 ± 0.16	0.46 ± 0.07	**0.029**	0.45 ± 0.05	0.38 ± 0.11	0.220	0.45 ± 0.04	0.36 ± 0.05	**0.009**
	K	0.43 ± 0.05	0.37 ± 0.04	**<0.001**	0.30 ± 0.07	0.19 ± 0.03	**0.015**	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	**0.003**	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	**<0.001**	0.35 ± 0.05	0.22 ± 0.02	**0.002**	0.26 ± 0.05	0.15 ± 0.02	**0.008**	0.27 ± 0.03	0.18 ± 0.03	**<0.001**

3.3.4 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 3.5. 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 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 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 3.5). 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.

Table 3.5 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.

		Exp-1						**Exp-2**					
(mg/g FW)		**53 DAS**			**43 DAS**			**50 DAS**			**57 DAS**		
		15 °C	**20 °C**	***p*-Value**	**10 °C**	**20 °C**	***p*-Value**	**10 °C**	**20 °C**	***p*-Value**	**10 °C**	**20 °C**	***p*-Value**
Leaves	Glucose	1.18 ± 0.35	1.44 ± 0.45	0.083	1.87 ± 0.52	0.52 ± 0.12	**0.004**	2.83 ± 1.22	0.82 ± 0.08	**0.021**	3.50 ± 1.43	1.41 ± 0.46	**0.014**
	Fructose	1.78 ± 0.65	2.10 ± 0.47	0.133	1.00 ± 0.52	0.27 ± 0.01	**0.034**	2.25 ± 1.20	0.48 ± 0.21	**0.028**	3.51 ± 1.18	1.43 ± 0.34	**0.006**
	Sucrose	1.38 ± 0.19	0.99 ± 0.20	**<0.001**	2.20 ± 0.58	0.58 ± 0.17	**0.002**	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	**0.038**	2.84 ± 0.13	2.30 ± 0.10	**<0.001**	5.84 ± 0.30	4.43 ± 0.57	**0.003**	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	**0.017**	1.31 ± 0.49	0.55 ± 0.24	**0.011**
Stems	Glucose	7.81 ± 0.99	7.64 ± 1.00	0.649	8.27 ± 1.41	3.78 ± 0.53	**0.001**	7.43 ± 0.90	5.83 ± 1.25	0.052	9.37 ± 1.46	7.21 ± 0.77	**0.013**
	Fructose	6.57 ± 0.82	6.49 ± 0.80	0.788	3.40 ± 1.45	1.04 ± 0.27	**0.020**	6.86 ± 0.72	4.62 ± 0.77	**0.001**	8.06 ± 0.88	6.29 ± 0.76	**0.004**
	Sucrose	1.91 ± 0.56	1.98 ± 0.53	0.697	7.50 ± 1.18	4.14 ± 0.80	**0.001**	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	**<0.001**	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

3.3.5 Effects of root temperature on glucosinolates

Six aliphatic glucosinolates were detected in both stems and leaves of Chinese broccoli in two experiments (Table 3.6). 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 3.6, 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 higher 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 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 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 3.6 Total, aliphatic, indolic and individual glucosinolates concentrations (μmol/100g 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	*p*-value	10 °C	20 °C	*p*-value	10 °C	20 °C	*p*-value
Leaves	Aliphatic	Sinigrin	14.66 ± 7.02	12.71 ± 7.15	0.506	15.35 ± 3.22	6.37 ± 1.91	**0.002**	18.11 ± 4.98	10.49 ± 3.65	**0.014**
		Progoitrin	1.39 ± 0.55	1.88 ± 1.54	0.322	1.46 ± 0.18	0.57 ± 0.06	**<0.001**	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	**0.001**	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	**0.002**	57.05 ± 20.01	41.17 ± 18.87	0.188
		Glucoiberin	1.77 ± 0.86	0.82 ± 0.56	**0.005**	0.43 ± 0.12	0.18 ± 0.08	**0.009**	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	**0.012**	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	**0.002**	84.00 ± 26.66	58.33 ± 24.98	0.116
	Indolic	Glucobrassicin	5.12 ± 2.80	4.66 ± 2.51	0.678	16.36 ± 7.97	5.79 ± 1.62	**0.040**	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	**0.036**	0.58 ± 0.15	0.49 ± 0.07	0.201
		Neoglucobrassicin	0.21 ± 0.14	0.09 ± 0.09	**0.024**	0.79 ± 0.31	0.27 ± 0.12	**0.017**	0.42 ± 0.18	0.23 ± 0.05	**0.044**
		4-Hydroxyglucobrassicin	1.15 ± 0.26	1.08 ± 0.27	0.502	0.97 ± 0.27	0.50 ± 0.07	**0.017**	1.49 ± 0.44	0.77 ± 0.23	**0.008**
		Total	7.01 ± 3.07	6.33 ± 2.69	0.570	18.64 ± 8.50	6.90 ± 1.70	**0.035**	12.90 ± 3.98	8.44 ± 2.65	**0.049**
	Total		58.84 ± 29.33	57.89 ± 35.68	0.944	84.25 ± 22.02	33.63 ± 9.44	**0.004**	96.90 ± 30.10	66.78 ± 27.55	0.101
Stems	Aliphatic	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	**0.018**	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	**0.025**	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
	Indolic	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	**0.031**	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

3.4 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 (Cao, 2004). 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 (Cao, 2004). 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 & Yang (2002) 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 (Sakamoto & Suzuki, 2015b). Possible mechanisms include alteration of turgor pressure, phytohormone production and altered cell wall properties (De Cnodder et al., 2005). The increased root biomass in Exp-1 may be explained by lower metabolic and respiration rates at low root temperature (He et al., 2009), 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. (2008) 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 3.3). We were unable to find published reports about the optimal root temperature for Chinese broccoli. The reduction of root dry biomass proved that 10 ℃ 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 (Bumgarner et al., 2012). Yan et al. (2013) showed that cucumber seedlings with roots immersed in a 12 ℃ nutrition solution had less total plant dry weight than seedlings with roots at 20 ℃. At 15 ℃ root temperature, *Valerianella locusta* plants had smaller shoots than plants at 20 ℃ (Dalla Costa et al., 2011). Exposure of red leaf lettuce to low root temperature (10 ℃) significantly reduced the fresh weights of the shoots (Sakamoto & Suzuki, 2015a). 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 (Aroca et al., 2001; Schwarz et al., 2010). Root phytohormone production including ABA could be altered by temperature, influencing shoot to root hormone signaling and shoot production (Aloni et al., 2010). Paul & Foyer (2001) 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 ℃) in Exp-2. The lower shoot ratio of both experiments indicated that root cooling channeled more photoassimilates to the growth of the root. Equiza (2001) 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. (2009)

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 (Starck et al., 2000). 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 (1995), 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. (2010) found that at sup-optimal root temperature, the roots of African snake tomato contained higher amounts of Ca. In contrast with Adebooye et al. (2010), 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 (Olle & Bender, 2009). 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 3.4). Mineral concentrations in the shoot are dependent on two processes: the uptake by roots and translocation from roots to shoots (Baghour et al., 2002). 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 (Rosa et al., 2001). Our results of carbohydrate concentration are consistent with the work of Rosa et al. (2011), 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 (He et al., 2009), red leaf lettuce (Sakamoto & Suzuki, 2015a), spinach (Chadirin et al., 2011), and cocktail tomato (He et al., 2019). 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 (Sami et al., 2016).

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 (León-Chan et al., 2017; Adebooye et al., 2010). 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 (Guy et al., 2008). ROS can cause chloroplast damage and therefore reduce chlorophyll levels (Ensminger et al., 2006). However, Gazula et al. (2005) reported that chlorophyll increased in three lettuce cultivars at lower air temperatures. Also, Kalisz et al.(2016) 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 (Zhang et al. 2008), 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 (Adebooye et al., 2010; Gazula et al., 2005). High root temperature stress reduced the amount of chlorophyll concentration in the leaves of carrot (Sakamoto & Suzuki, 2015b). In the experiments of Sun et al. (2016) with lettuce (*Lactuca sativa* L.) and Nguyen et al. (2020) 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. (2016) 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 (Sun et al., 2016). 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 (Sun et al., 2012 a & b; La et al., 2009). 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 (Sun et al., 2011). 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 (Martínez-Ballesta et al., 2013).

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 (Soydam Aydin et al., 2013; Schulz et al., 2016). Engelen-Eigles et al. (2006) 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 (Kissen et al., 2016). Similarly, in *Brassica oleracea* L, higher glucosinolate levels in leaves were obtained when plants were moved from 32 to 12 °C compared to being kept at a constant 32 °C (Charron & Sams, 2004). On the other hand, Guo et al. (2014) 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 & Rodrigues (1998) 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 (Steindal et al., 2015). 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 (Cocetta et al., 2018). This may have been due to breakdown of glucosinolates upon tissue damage caused by heating (Kask et al., 2016). 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 (Farnham et al., 2004). However, previous studies on *Arabidopsis thaliana* (Charron & Sams, 2004) and kale (Steindal et al., 2015), 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. (2016) 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. (Soydam Aydin et al., 2013) observed that short-term responses of tomato to cold maximised antioxidant enzyme, which was present after 22 days. Ntatsi et al. (2017) and Yang et al. (2015) 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. (2009), 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 gradually decline in differences between the two groups in Exp-2 here also supports conclusions of previous studies.

Chapter 4

Effects of short-term root cooling before harvest on yield and food quality of Chinese broccoli (*Brassica oleracea* var. *alboglabra* Bailey)

He, F., Thiele, B., Kraus, D., Bouteyine, S., Watt, M., Kraska, T., ... & Kuhn, A. J. (2021). Effects of short-term root cooling before harvest on yield and food quality of Chinese broccoli (*Brassica oleracea* var. *alboglabra* Bailey). *Agronomy*, *11*(3), 577. https://doi.org/10.3390/agronomy11030577

4.1. 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 (Guo et al., 2018). Chinese broccoli is grown for its bolting stems and tender rosette leaves as the main edible parts (Sun et al. 2012). 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 (Sun et al. 2012). Notably, glucosinolates are the characteristic natural antioxidants in Brassica vegetables and a group of sulfur-and nitrogen-containing compounds derived from different amino acids (Del Carmen Martínez-Ballesta et al., 2013). 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 (Dinkova-Kostova & Kostov, 2012; Fahey et al., 2012). Sugar levels, together with glucosinolates, determine the flavor and acceptance of Chinese broccoli by the consumer (Rosa et al. 2001). 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 (Kawashima & Valente Soares 2003).

Root temperature plays a critical role in plant growth and influences the concentration of some primary and most secondary metabolites (Islam et al., 2019; Ito & Shimizu, 2020; Sakamoto & Suzuki, 2015a, 2015b). 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 (Variyar et al., 2014). 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 (Lam et al., 2020). In hydroponically grown carrots, a 14-day treatment with an elevated nutrient solution temperature increased the total phenolic compounds and soluble solid content (Sakamoto & Suzuki, 2015a). Similarly, our previous studies of the effects of root temperature on food quality of Chinese broccoli (He et al., 2020) and cocktail tomato (He et al., 2019) 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 (Hwang et al., 2016; Kayum et al., 2016). For example, long-term root cooling caused 8–21% yield loss in cocktail tomato (He et al., 2019) and 21% loss in Chinese broccoli (He et al., 2020). Under long-term cold stress conditions, photosynthesis, root respiration, water uptake, and hormone signaling were altered (Aroca et al., 2001; Eremina et al., 2016; Schwarz et al., 2010). Additionally, the reallocation of resources to accumulate phytochemicals was at the expense of growth (Ramakrishna & Ravishankar, 2011), resulting in a reduced vegetable yield—an undesirable outcome for growers. Ogawa et al. (2018) 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 (Chadirin et al., 2011). 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 (He et al., 2020). As Chinese broccoli is grown all year round and under diverse climatic conditions, products may have different phytochemical concentrations as a result (Rosa & Rodrigues, 1998). To account for the climatic factors, we conducted two separate experiments in summer (Exp-3) and autumn (Exp-4). 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.

4.2. Materials and Methods

4.2.1 Plant materials and experimental setup

Experiments were conducted under greenhouse conditions (Forschungszentrum Jülich, Germany), in August 2018 (Exp-3) and October 2019 (Exp-4). 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) (Füllner et al., 2012). On the third true leaf stage (31 DAS), 32 uniform seedlings were transplanted into 8 containers (28 × 43 × 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 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) (Sun et al., 2012). 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,

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

4.2.2 Evaluation of photosynthesis, transpiration, stomatal conductance and leaf temperature

In Exp-4, net photosynthesis, transpiration, stomatal conductance and leaf temperature were determined by a portable photosynthesis system, LI-6400 (LiCor, Lincoln, Nebraska, 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-3 were missed.

4.2.3 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 (Viola & Davies, 1992) with slight modifications described by He et al. (2019). 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, Vermont, USA) at 652 nm. Glucose, fructose and sucrose concentrations were determined at 340 nm based on Viola and Davies (Viola & Davies, 1992) with slight modification described by He et al. (2019). 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.

4.2.4 Mineral elements quantification

Dried Chinese broccoli leaf, stem samples of Exp-3 and Exp-4 and root samples of Exp-4 were finely ground in a pebble mill (MM400, Retsch, Haan, Germany). Root samples of Exp-3 were not analyzed due to mishandling during the preparation. Carbon and nitrogen were analyzed with a CHNS-Analyzer (Leco CHNS-932, St. Joseph, Missouri 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..

4.2.5 Glucosinolates analysis

Intact individual glucosinolates were determined as described by He et al. (2020). Briefly, approximately 60 mg of homogenized frozen leaf and stem samples of Exp-3 and Exp-4 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, 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 based on 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.

4.2.6 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 ± 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.

4.3 Results

4.3.1 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 4.1). Fluctuations of air temperature and light were consistent for each experiment, but average daily air temperature and light during Exp-4 were lower than those of Exp-3.

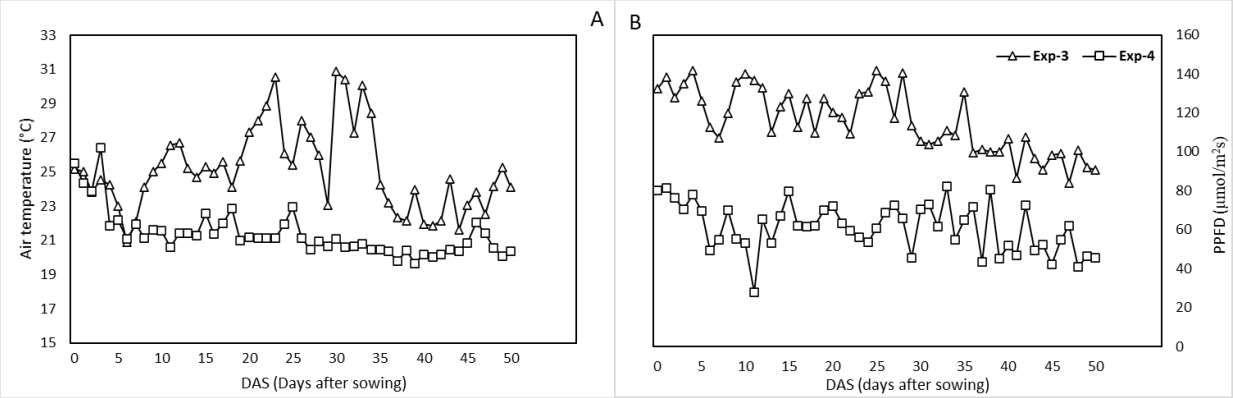

Figure 4.1 Daily average air temperature (**A**) and PPFD (photosynthetic photon flux density) (**B**) recorded during Exp-3 and Exp-4 to test the influence of rooting temperature on Chinese broccoli growth and quality in a glasshouse. DAS: days after sowing.

4.3.2 Biomass and yield

Table 4.1. 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-3 and Exp-4. Significant differences ($p < 0.05$) are indicated in bold.

	Experiment	**Exp-3**			**Exp-4**		
	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

In both experiments, only the fresh weight of shoots was significantly affected by the root temperature (Table 4.1). 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-4 was reduced by 18.9% ($p =$

0.009), while no differences were observed in Exp-3. 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.

4.3.3 Net photosynthesis, transpiration, stomatal conductance and leaf temperature

In Exp-4, net photosynthesis, transpiration and stomatal conductance were recorded three days (47 DAS) and six days (50 DAS) after the treatment started (Table 4.2) 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 4.2 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-4. Significant differences ($p < 0.05$) are indicated in bold.

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

4.3.4 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 4.3). 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-3. In Exp-4, 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-3, no significant changes in stem sugar levels were observed in root cooling-treated plants, while in Exp-4, 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-4, with a 68.8% increase. In Exp-3, the concentration of total chlorophyll in leaves increased by around 5.8% at a root temperature of 10 °C compared to 20 °C (Table 4.3).

Table 4.3 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-3 and Exp-4. Significant differences ($p < 0.05$) are indicated in bold. FW: fresh weight.

		Exp-3				Exp-4			
(mg/g FW)	**Treatment**	**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**

4.3.5 Glucosinolates

The glucosinolate patterns of the stems and leaves were similar in both Exp-3 and Exp-4 (Table 4.4). 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-3, 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-4 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.

Table 4.4 Total, aliphatic, indolic and individual glucosinolates concentrations (μmol/100g FW) in the leaves and stems of Chinese broccoli under different root temperatures and harvest dates in Exp-3 and Exp-4. Significant differences ($p < 0.05$) are indicated in bold. FW: fresh weight.

(μmol/100g FW)			Exp-3				Exp-4			
		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

4.3.6 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 4.5). Under root cooling in Exp-3, 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-4, 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-4 was significantly affected for N only (Figure 4.2). 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.

Table 4.5 Element concentration (% DW) of leaves, stems and roots of Chinese broccoli from different root temperatures and harvest dates in Exp-3 and Exp-4. Significant differences ($p < 0.05$) are 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.

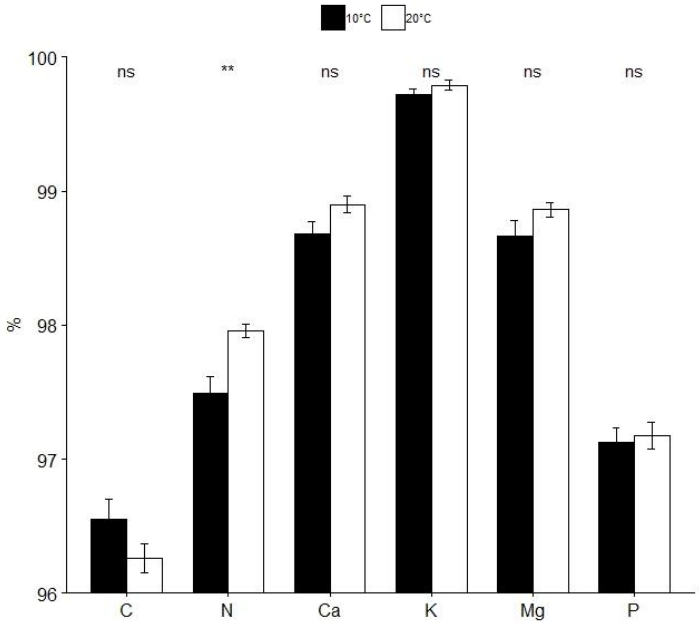

Figure 4.2 Shoot/root C, N, Ca, K, Mg, P content ratio of plants grown at two root temperature treatments (10 and 20°C) in Exp-4. Mean and standard deviation are shown. Statistical analysis was performed by t-test. Levels of significance are represented by ns not significant, 0.001<∗∗ $p < 0.01$.

4.4 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 (Yang & Yang, 2002).

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. (2010) 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 (Lam et al., 2020). 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 (Sakamoto & Suzuki, 2015a). 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 (Hao et al., 2012), a hormone signaling imbalance during the cooling treatment (Aloni et al., 2010), or reduced photosynthesis (Aroca et al., 2001; Schwarz et al., 2010). However, shoot dry weight was not affected by root cooling, which indicated that the shoot water status was influenced by root temperature (Dodd et al., 2000). The balance between root water uptake and shoot transpiration determines the shoot's water status (Nagasuga et al., 2011). A higher transpiration rate was detected in the plants exposed to cooling temperatures in Exp-4 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 Poiré et al. (2010), 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 Poiré et al. (2010) 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 (Kuwagata et al., 2012; Nagasuga et al., 2011) and related this to the simultaneously reduced root water uptake and subsequent carbon assimilation (Wang et al., 2016). No consensus on root temperature effects has been achieved to date, possibly due to differences in species, treatment patterns, cultivation systems and study location (Wang et al., 2016). 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 (Taranet et al., 2018). 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 (Aidoo et al., 2019). The long-term effects of low root temperature include cold acclimation of nutrient uptake, root respiration, photosynthesis and transpiration (Atkin et al., 2000), therefore, the impact on biomass might be more complicated. In this study, the 18.9% reduction in yield was only observed in Exp-4 and lower than a previously documented 20.6% decrease in yield after long-term root cooling (He et al., 2020). 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 (Hao et al., 2012). 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. (2012) and Shimono et al., (2004), who reported that the photosynthetic rate of rice was not affected by the low temperature of the nutrient solution. Moreover, Nagasuga et al. (2011) 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-4 were improved at the lower root temperature, which could be due to the alteration of the water status (Dodd et al., 2000), while the lack of differences in these two parameters at 50 DAS between the two groups could be indicative of cold acclimation (Atkin et al., 2000).

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 (Rosa et al., 2001). The concentrations of three soluble sugars in stems were higher in comparison to leaves, consistent with previous studies (Wang et al., 2017). Starch is the primary storage component (Liu et al., 2018), 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 (Arai-Sanoh et al., 2010). The increase of carbohydrate concentration, especially in Exp-4, 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 (Poiré et al., 2010). In red leaf lettuce, a 7-day low root temperature treatment

accelerated the accumulation of sugars (Sakamoto & Suzuki, 2015a). These accumulated sugars may act as osmolytes to maintain turgor pressure, substrates for plants to survive stress and antioxidants to scavenge ROS (Sami et al., 2016). 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 (Peuke et al., 2006). Chadirin et al. (2011) 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-3 was consistent with our previous study (He et al., 2020). In contrast, Adebooye et al., (2010) 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., (2019) demonstrated that the chlorophyll content of cucumber seedlings was significantly reduced at a low root temperature (14 °C). Considering the warm conditions in Exp-3, 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 (Bumgarner et al., 2012). Therefore, the impacts of root temperature on chlorophyll depend on other abiotic factors, the intensity of temperature stress and cultivars (Anwar et al., 2019).

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 (Sun et al. 2012). Based on previous studies, the total

glucosinolates of Chinese broccoli include aliphatic, indolic and aromatic groups (La et al., 2008 & 2009). However, no aromatic glucosinolates could be detected in our study. Rosa and Rodrigues (Rosa & Rodrigues, 1998) 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 (Rosa & Rodrigues, 1998). 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 (Variyar et al., 2014). 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 & Sams (2004) and Steindal et al. (2013) reported a higher concentration of total glucosinolates in leaves of broccoli and kale at lower ambient temperature, while, Rosa and Rodrigues (Rosa & Rodrigues, 1998) 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 (Del Carmen Martínez-Ballesta et al., 2013; Engelen-Eigles et al., 2006; Farnham et al., 2004).

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 (Cocetta et al., 2018). 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 (He et al., 2020), especially indolic glucosinolates. However, research on *Arabidopsis thaliana* (Kissen et al., 2016) and kale (Steindal et al., 2015) 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 (Kissen et al., 2016). 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-4. 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 (Adebooye et al., 2010). As a result of root cooling stress, hydraulic conductivity may be reduced, causing the uptake rate to be impeded (Baghour et al., 2003). 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 (Pettersson, 1995). 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-4 was likely caused by the

slow sink metabolism and cold girding effect on the phloem pathway (Poiré et al., 2010). We also calculated elemental ratios (% based on DW) in Exp-4 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 (Starck et al., 2000). Aidoo et al. (2017) 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. (2017), our results in Exp-4 showed that nitrogen tended to accumulate in the roots, while other elements were unaffected.

Chapter 5

Taste of cocktail tomato (*Lycopersicum esculentum* Mill.) as influenced by lowering root temperature

5.1 Introduction

Sensory properties play an essential role in assessing fruit and vegetable quality by consumers and their purchase behavior (Tronstad, 1995). Only the products meeting the consumer expectations can survive in the market. Cocktail tomatoes are usually consumed raw and fresh, and therefore they should have palatable color, flavor, aroma and texture characteristics. The flavor of tomato is largely determined by the sugar, acids and the ratio of sugar to acids (Malundo et al., 1995). For consumers and the food industry, sweetness and acidity are regarded as essential quality factors (Kader 2008). The major sugars of tomato are glucose and fructose with trace amounts of sucrose, and organic acids are primarily citric acid and malic acid (Selli et al., 2014). Compared to normal-sized tomatoes, cocktail tomatoes are characterized by relatively higher sugar concentration and intensity of aroma (Causse et al., 2001).

For the intensive production of tomatoes in the greenhouse, soilless cultivation systems are used. A practical possibility to influence fruit quality is to change the temperature of the nutrient solution. The soluble sugar concentration of cocktail tomatoes was proved to be increased at lower root temperatures in our previous report (He et al., 2019). Many other investigations have shown that altering root temperatures of greenhouse horticultural plants in soilless culture produces vegetables and fruits with a higher concentration of sugars, organic acids and antioxidants (Sakamoto & Suzuki, 2015 a&b). However, it is not clear that the effect always improves the sensory properties of the fruits. Hence, to produce high-quality fruit, research about the quantitative and qualitative sensory changes of the product and the product's consumer preferences under root cooling treatments is necessary. In this chapter, the effects of root cooling on the sensory aspects and consumer preference of two cocktail tomato cultivars are evaluated and reported from a human taste-testing experiment.

5.2 Materials and Methods

5.2.1 General treatment conditions

The cultivation experiment was carried out under greenhouse conditions in the hydroponic system from the 13th of September, 2019 to the 15th of February, 2020 using two cocktail tomatoes (*Lycopersicum esculentum* Mill.) cultivars, 'Amoroso' and 'Delioso'. Cultivation details were the same as Chapter 2 (He et al., 2019). Briefly, 12 plants per cultivar were grown randomly in two rows. A root temperature treatment of 10°C versus control (16-27°C) was initiated after the second inflorescence. Shoots above the 7th cluster were topped off. Old leaves below the turning clusters were removed. Side shoots were removed twice a week. Pollination was carried out by an electronic toothbrush twice a week after the first flower appeared.

5.2.2 Sensory evaluations

Eight proximal fruits of the fourth cluster of both cultivars and two root temperature treatments at the red-ripe stage were harvested and washed at 9:00 a.m. on the 5th and 19th of February, 2020. Fruits from each cultivar and each treatment were regarded as groups A, B, C, D. There were 48 fruits in each group (6 plants in each treatment and each cultivar). Based on the fruit position within the same cluster, the group was further divided into eight subgroups, such as A1, A2, A3, A4, A5, A6, A7, A8. The fruits were put into the box marked with the responding position, e.g., A1. The weight of the fruits was measured and recorded.

The sensory investigation was conducted twice in the format of descriptive analysis and preference testings. Twenty volunteers were randomly selected at IBG-2 (Plant Sciences) of Juelich Forschungszentrum twice and served as the taste panel. Some of the volunteers

were repeated in two tests. In all sensory tests, the assessors received two whole cocktail tomato fruits for the evaluation and worked separately to avoid discussion. The volunteers assessed the fruits for the first impression, aroma, peel hardness, fruit sweetness, acidity and overall. Two fruits from the same subgroup were presented in plastic cups labeled with "A, B, C, D" on a tray. Each volunteer evaluated the fruits from two root temperature treatments and two cultivars of the same position on the cluster, e.g. A1, B1, C1, D1 to avoid the effects of fruit position. Between two samples, stilled mineral water and white bread were provided to clear the palates.

Table 5.1 Taste-testing form of sensory evaluation - Descriptive analysis.

	A	**B**	**C**	**D**
Hardness: How does it feel at the first bite?				
Sweetness				
Acidity				
Aroma: How does it smell?				
Overall preference: How would you rate the tomato overall?				

Choose the number 1-5 that best matches. 1: Dislike, 5: Like

- Descriptive analysis

The panelists were provided the taste assessment form (Table 5.1) to grade peel hardness, fruit sweetness, acidity, aroma and overall preference as 1 to5, 1 as " the weakest", 5 as "the strongest".

- Preference testing

Based on the first experiment, a second taste test of the six clusters was conducted on 19 February 2020. The taste testing form (Table 5.2) was provided to the assessors to select the preferred cocktail tomato group in each attribute of fruit quality.

Table 5.2 Taste-testing form of sensory evaluation - Preference testing

	A	B	C	D
Hardness				
Sweetness				
Acidity				
Aroma				
Overall preference				

Cross the one that you think is better. A vs. B and C vs. D.

5.2.3 Statistical analysis

In the first test, all data were statistically analyzed by Student T-test. Statistical analysis was done using the R software. Differences at $p \leqq 0.05$ were considered statistically significant and were indicated in *. In the second test, the number of preferences was listed in the table as the results.

5.3 Results and Discussion

5.3.1 Descriptive analysis

Figures 5.1 and 5.2 indicated the taste-testing process and how it was conducted. Figure 5.3 summarises the differences in five sensory characteristics of two cultivars and two root temperature treatments investigated. Root temperature affected different attributes in each cultivar. The results of 'Amoroso' showed no significant differences in the sensory

evaluations between the control and cool group, either in flavor or taste. One possible reason could be the limited number of sampling, given only two fruits were provided to test. For 'Delioso', all the values showed an increasing trend in the cooling root group. However, only in aroma, the results were significantly different. Sweetness is positively correlated with sugar levels, and acid concentration had a positive correlation with the sour taste (Malundo et al., 1995). Though the glucose and fructose levels were increased at lower root temperature (He et al., 2019), the increased amount may not achieve the detectable threshold. The aroma of fresh ripe tomato can be attributed to a complex mixture of over 400 different volatile compounds, such as aldehydes, alcohols, esters, ketones, sulfurs and volatiles, which are mostly derived biosynthetically from the degradation of fatty and amino acids, terpenes and carotenoids (Davidovich-Rikanati et al., 2016; Selli et al., 2014). Studies have found that carotenoid breakdown is related to aroma volatility (Davidovich-Rikanati et al., 2016). For example, lycopene gives rise to non-cyclic volatiles neural and geranial (Lewinsohn et al., 2005). Considering the increased lycopene concentration in our previous report (He et al., 2019), the improved aroma could be explained.

Figure 5.1 Volunteers testing the cocktail tomato samples.

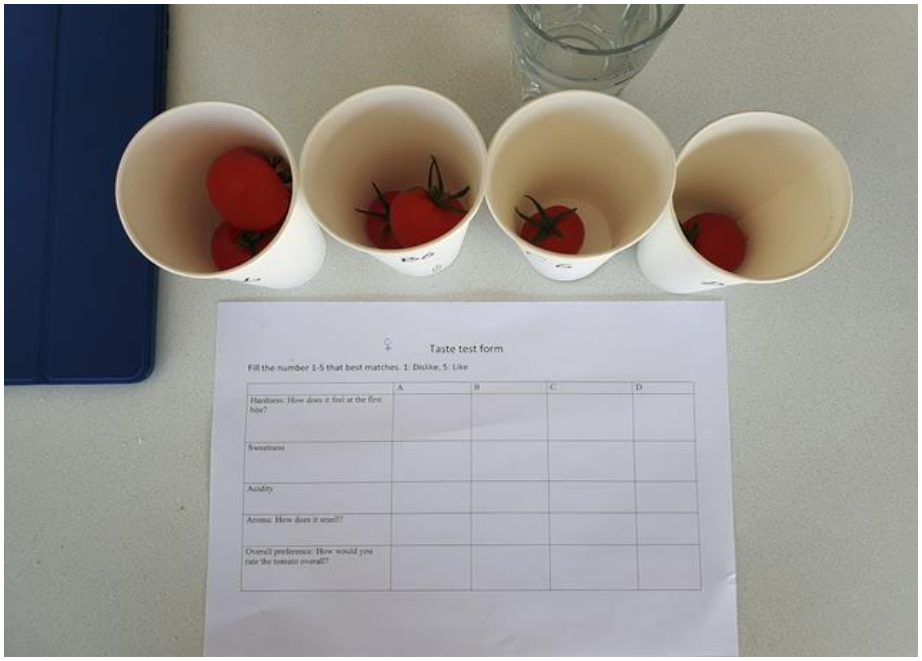

Figure 5.2 Display of the cocktail tomato sample and taste test forms of the descriptive analysis.

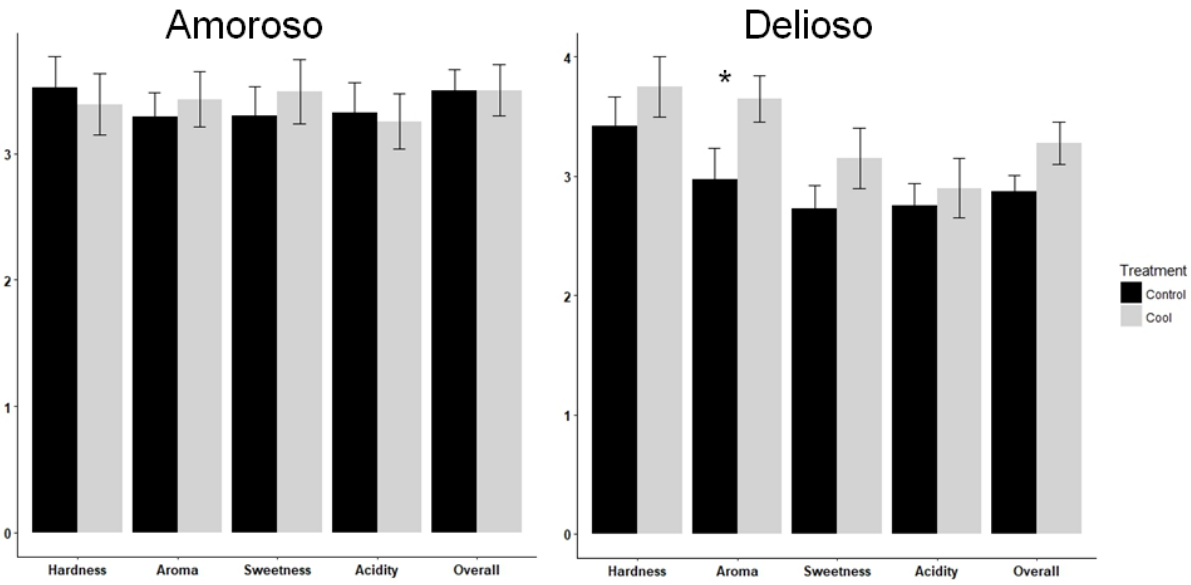

Figure 5.3 Results of descriptive analysis between two root temperature treatments and two cultivars.

Table 5.3 Results of preference testings between root temperature treatments within one cultivar 'Amoroso' or 'Delioso'. Numbers indicated the number of volunteers who voted.

Cultivar	'Amoroso'		'Delioso'	
Treatment	Cool	Control	Cool	Control
Aroma	14	6	11	11
Hardness	12	8	15	8
Sweetness	10	10	14	6
Acidity	11	9	10	11
Overall	12	8	13	9

5.3.2 Preference testing

Based on the results of the first descriptive analysis, we conducted the second taste assessment, but in the form of preference testing. Since the volunteers were not trained, preference testing is more likely to provide the differences between the two samples. Since our focus was on the root temperature treatment, only the preference within the same cultivar was selected.

The results of the second test were promising and distinctive. The number of volunteers chosen for the aroma, hardness, acidity and overall values of 'Amoroso' was larger in the cooling group. However, in the first descriptive analysis test, no differences were found. For 'Delioso', the differences between cool and control groups were even larger, especially for the factor of hardness and sweetness, in which the number of preferences in the cool group was double that of the control group. Sweetness is one of the critical components for consumers, and the differences in sweetness attributes were in line with the previous chemical analysis results of glucose and fructose (He et al., 2019). The hardness of the tomato skin determines the shelf life and commercial value, and therefore, is the desired

quality by the producers (Liu et al., 2010). However, the significant differences in the aroma of 'Delioso' during the first test were not detected by the volunteers in the second test, which also reflected the differences between these two test forms. In addition, the different fruit positions of the two taste assessments could also explain the different results.

Chapter 6

Conclusion and outlook

6.1 Conclusion

The objective of the thesis was to obtain a better understanding of the effects of root temperature on plant growth and food quality of horticultural crops by reducing the root temperature. We aimed to improve the consumer-oriented character of vegetables and fruits without negatively influencing the yield.

In the first paper (He et al., 2019) discussed in Chapter 2 of the thesis, we applied 10°C vs. 20°C root temperature after the second inflorescences in two cocktail tomato genotypes. Taking the seasons into account, we assessed two cultivations in 2017 winter and 2018 summer. The results showed that root cooling could improve various qualities of cocktail tomatoes, such as glucose, fructose, and Vitamin C concentration based on the fresh weight (mg/g FW). However, the effect was dependent on the season, especially the ambient temperature and light intensity. Yield, which is the most important factor for farmers, was reduced to approximately 20% in 'Delioso' in winter. However, in summer, root cooling alleviated the hot stress from ambient temperature and enhancing the yield.

Our experiments from the second paper (He et al., 2020) in Chapter 3 of the thesis shifted to the leafy vegetable Chinese broccoli, and the root temperature was maintained as 15°C vs. 20°C and 10°C vs. 20°C in autumn 2017 and spring 2018. The treatment started two days after transplant and lasted until the harvest. 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. At 10 °C root temperature, the levels of soluble sugars and glucosinolates in the shoots were strongly enhanced, but the yield was simultaneously reduced compared to 20°C. Because these parameters correlate with the sensory descriptors of good flavour and healthy parameters, a lower root temperature most likely has a positive effect on Chinese broccoli taste and antioxidants levels.

In the third manuscript of Chapter 4 in the thesis, 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 3. 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

In Chapter 5, we used a panel of human testers to review the taste of cocktail tomatoes grown under two root temperatures (10 and 20°C) using two methods: descriptive analysis and preference testings. Since the participants were untrained in tasting, the descriptive analysis did not reveal taste differences between growth temperature treatments. Preference testing was aimed at normal consumers, and the results were in high agreement with previous chemical analysis in Chapter 2 (He et al., 2019). This sensory evaluation further supported that root cooling can be used to improve the quality of cocktail tomato, without negative taste effects.

6.2 Outlook

The next steps would be to

i. find the best combination of root temperature, treatment duration, air temperature and light intensity in the greenhouse to achieve the best qualities and further minimize the reduction in the yield;

ii. explore the physiological mechanisms of the changes in food quality attributes under different root temperatures, such as molecular regulation of the chemical components changes, water and nutrient uptake and distribution among different plant organs, photosynthesis and transpiration, interaction between water relations and carbohydrate metabolisms, etc;

iii. conducte a similar study to this thesis with more pharmaceutical or medical plants to identify beneficial and valuable compounds that can be controlled with root temperature.

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References

Abdel-Mawgoud, A. M. R., Sassine, Y. N., El-Behairy, U. A., Abou-Hadid, A. F., & El-Abd, S. O. (2005). Effect of minimum root-zone temperature on the growth and production of greenhouse sweet pepper. *Journal of Applied Sciences Research*, *1*, 72–77.

Achard, P., Gong, F., Cheminant, S., Alioua, M., Hedden, P., & Genschik, P. (2008). The cold-inducible CBF1 factor-dependent signaling pathway modulates the accumulation of the growth-repressing DELLA proteins via its effect on gibberellin metabolism. *The Plant Cell*, *20*(8), 2117–2129.

Adalid, A. M., Roselló, S., & Nuez, F. (2010). Evaluation and selection of tomato accessions (*Solanum section Lycopersicon*) for content of lycopene, β-carotene and ascorbic acid. *Journal of Food Composition and Analysis*, *23*(6), 613–618.

Adams, S. R., Cockshull, K. E., & Cave, C. R. J. (2001). Effect of temperature on the growth and development of tomato fruits. *Annals of Botany*, *88*(5), 869–877.

Adebooye, O. C., Schmitz-Eiberger, M., Lankes, C., & Noga, G. J. (2010). Inhibitory effects of sub-optimal root zone temperature on leaf bioactive components, photosystem II (PS II) and minerals uptake in *Trichosanthes cucumerina* L. Cucurbitaceae. In *Acta Physiologiae Plantarum*, *32*(1), 67-73

Ahamed, A., Murai-Hatano, M., Ishikawa-Sakurai, J., Hayashi, H., Kawamura, Y., & Uemura, M. (2012). Cold stress-induced acclimation in rice is mediated by root-specific aquaporins. *Plant & Cell Physiology*, *53*(8), 1445–1456.

Ahn, S.-J., Im, Y.-J., Chung, G.-C., Cho, B. H., & Suh, S. R. (1999). Physiological responses of grafted-cucumber leaves and rootstock roots affected by low root temperature. *Scientia Horticulturae*, *81*(4), 397–408.

Ahn, S.-J., Im, Y.-J., Chung, G.-C., Seong, K.-Y., & Cho, B.-H. (2000). Sensitivity of plasma membrane H^+-ATPase of cucumber root system in response to low root temperature. *Plant Cell Reports*, *19*(8), 831–835.

Aidoo, M. K., Bdolach, E., Fait, A., Lazarovitch, N., & Rachmilevitch, S. (2016). Tolerance to high soil temperature in foxtail millet (*Setaria italica* L.) is related to shoot and root growth and metabolism. *Plant Physiology and Biochemistry*, *106*, 73–81.

Aidoo, M. K., Sherman, T., Lazarovitch, N., Fait, A., & Rachmilevitch, S. (2017). 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. *Physiologia Plantarum*, *161* (2), 196–210.

Aidoo, M. K., Sherman, T., Lazarovitch, N., Fait, A., & Rachmilevitch, S. (2019). Physiology and metabolism of grafted bell pepper in response to low root-zone temperature. *Functional Plant Biology*, *46*(4), 339–349.

Aloni, B., Cohen, R., Karni, L., Aktas, H., & Edelstein, M. (2010). Hormonal signaling in rootstock–scion interactions. *Scientia Horticulturae*, *127*(2), 119–126.

Ameen, M., Zhang, Z., Wang, X., Yaseen, M., Umair, M., Noor, R. S., Lu, W., Yousaf, K., Ullah, F., & Memon, M. S. (2019). An investigation of a root zone heating system and its effects on the morphology of winter-grown green peppers. *Energies*, *12*(5), 933.

Anthon, G. E., LeStrange, M., & Barrett, D. M. (2011). Changes in pH, acids, sugars and other quality parameters during extended vine holding of ripe processing tomatoes. *Journal of the Science of Food and Agriculture*, *91*(7), 1175–1181.

Anwar, A., Di, Q., Yan, Y., He, C., Li, Y., & Yu, X. (2019). Exogenous 24-epibrassinolide alleviates the detrimental effects of suboptimal root zone temperature in cucumber seedlings. *Archives of Agronomy and Soil Science*, *65*(14), 1927–1940.

Apel, K., & Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology*, *55*, 373–399.

Arai-Sanoh, Y., Ishimaru, T., Ohsumi, A., & Kondo, M. (2010). Effects of soil temperature on growth and root function in rice. *Plant Production Science*, *13*(3), 235–242.

Araki, T., Kitano, M., & Eguchi, H. (2000). Dynamics of fruit growth and photoassimilate translocation in tomato plant (*Lycopersicon esculentum* Mill.) under controlled environment. *International Conference and British-Israeli Workshop on Greenhouse Techniques towards the 3rd Millennium*, *534*, 85–92.

Aroca, R., Tognoni, F., Irigoyen, J. J., Sánchez-Díaz, M., & Pardossi, A. (2001). Different root low temperature response of two maize genotypes differing in chilling sensitivity. *Plant Physiology and Biochemistry*, *39*(12), 1067–1073.

Atkin, O. K., Edwards, E. J., & Loveys, B. R. (2000). Response of root respiration to changes in temperature and its relevance to global warming: REVIEW Temperature and root respiration. *The New Phytologist*, *147*(1), 141–154.

Atkinson, N. J., Dew, T. P., Orfila, C., & Urwin, P. E. (2011). Influence of combined biotic and abiotic stress on nutritional quality parameters in tomato (*Solanum lycopersicum*). *Journal of Agricultural and Food Chemistry*, *59*(17), 9673–9682.

Badea, C., & Basu, S. K. (2009). The effect of low temperature on metabolism of membrane lipids in plants and associated gene expression. *Plant Omics*, *2*(2), 78.

Baghour, M., Moreno, D. A., Víllora, G., López-Cantarero, I., Hernández, J., Castilla, N., & Romero, L. (2002). Root-zone temperature influences the distribution of Cu and Zn in potato-plant organs. *Journal of Agricultural and Food Chemistry*, *50*(1), 140–146.

Baghour, M., Ragala, L., Moreno, D. A., Villora, G., Hernández, J., Castilla, N., & Romero, L. (2003). Effect of root zone temperature on accumulation of molybdenum and nitrogen metabolism in potato plants. *Journal of Plant Nutrition*, *26*(2), 443–461.

Bai, L., Deng, H., Zhang, X., Yu, X., & Li, Y. (2016). Gibberellin is involved in inhibition of cucumber growth and nitrogen uptake at suboptimal root-zone temperatures. *PloS One*, *11*(5), e0156188.

Barrero-Gil, J., Huertas, R., Rambla, J. L., Granell, A., & Salinas, J. (2016). Tomato plants increase their tolerance to low temperature in a chilling acclimation process entailing comprehensive transcriptional and metabolic adjustments. In *Plant, Cell & Environment*, *39*(10), 2303–2318.

Barten, J. H. M., Scott, J. W., Kedar, N., & Elkind, Y. (1992). Low temperatures induce rough blossom-end scarring of tomato fruit during early flower development. *Journal of the American Society for Horticultural Science*, *117*(2), 298–303.

Beauchamp, E. G., & Lathwell, D. J. (1967). Root-zone temperature effects on the early development of maize. *Plant and Soil*, *26*(2), 224–234.

Beauvoit, B. P., Colombié, S., Monier, A., Andrieu, M.-H., Biais, B., Bénard, C., Chéniclet, C., Dieuaide-Noubhani, M., Nazaret, C., Mazat, J.-P., & Gibon, Y. (2014). Model-assisted analysis of sugar metabolism throughout tomato fruit development reveals enzyme and carrier properties in relation to vacuole expansion. *The Plant Cell*, *26*(8), 3224–3242.

Beckles, D. M. (2012). Factors affecting the postharvest soluble solids and sugar content of tomato (*Solanum lycopersicum* L.) fruit. *Postharvest Biology and Technology*, *63*(1), 129–140.

Bertin, N., Génard, M., & Others. (2018). Tomato quality as influenced by preharvest factors. *Scientia Horticulturae*, *233*, 264–276.

Bloom, A. J., Zwieniecki, M. A., Passioura, J. B., Randall, L. B., Holbrook, N. M., & St. Clair, D. A. (2004). Water relations under root chilling in a sensitive and tolerant tomato species. *Plant, Cell & Environment*, *27*(8), 971–979.

Böhm, V. (2012). Lycopene and heart health. *Molecular Nutrition & Food Research*, *56*(2), 296–303.

Borguini, R. G., & Ferraz Da Silva Torres, E. A. (2009). Tomatoes and tomato products as dietary sources of antioxidants. *Food Reviews International*, *25*(4), 313–325.

Brandt, S., Lugasi, A., Barna, É., Hóvári, J., Pék, Z., & Helyes, L. (2003). Effects of the growing methods and conditions on the lycopene content of tomato fruits. *Acta Alimentaria*, *32*(3), 269–278.

Bugbee, B., & White, J. W. (1984). Tomato growth as affected by root-zone temperature and the addition of gibberellic acid and kinetin to nutrient solutions. *Journal of the American Society for Horticultural Science*, *109*(1), 121–125.

Bumgarner, N. R., Scheerens, J. C., Mullen, R. W., Bennett, M. A., Ling, P. P., & Kleinhenz, M. D. (2012). Root-zone temperature and nitrogen affect the yield and secondary metabolite concentration of fall-and spring-grown, high-density leaf lettuce. *Journal of the Science of Food and Agriculture*, *92*(1), 116–124.

Campos Menezes, J. B. (2012). Fruit production and classification of four cherry tomato genotypes under an organic cropping system. *Idesia*, *30*(3):29-35.

Cao H. (2004). Practical cultivation technique of Chinese kale, Choy sum, Wild cabbage and leaf mustard. China Agricultural Press.

Casals, J., Rivera, A., Sabaté, J., Romero del Castillo, R., & Simó, J. (2018). Cherry and fresh market tomatoes: differences in chemical, morphological, and sensory traits and their implications for consumer acceptance. *Agronomy*, *9*(1), 9.

Causse, M., Saliba-Colombani, V., Lesschaeve, I., & Buret, M. (2001). Genetic analysis of organoleptic quality in fresh market tomato. 2. Mapping QTLs for sensory attributes. *Theoretical and Applied Genetics*, *102*(2), 273–283.

Chadirin, Y., Hidaka, K., Takahashi, T., Sago, Y., Wajima, T., & Kitano, M. (2011). Application of temperature stress to roots of spinach i. effect of the low temperature stress on quality. *Environmental Control in Biology*, *49*(3), 133–139.

Chapin, F. S., 3rd. (1974). Phosphate absorption capacity and acclimation potential in plants along a latitudinal gradient. *Science*, *183*(4124), 521–523.

Charron, C. S., & Sams, C. E. (2004). Glucosinolate content and myrosinase activity in rapid-cycling *Brassica oleracea* grown in a controlled environment. *Journal of the American Society for Horticultural Science*, *129*(3), 321–330.

Chen, X., Wu, J., Zhou, S., Yang, Y., Ni, X., Yang, J., Zhu, Z., & Shi, C. (2009). Application of near-infrared reflectance spectroscopy to evaluate the lutein and β-carotene in Chinese kale. *Journal of Food*

Composition and Analysis, *22*(2), 148–153.

Cheung, K. L., & Kong, A.-N. (2010). Molecular targets of dietary phenethyl isothiocyanate and sulforaphane for cancer chemoprevention. *The AAPS Journal*, *12*(1), 87–97.

Choi, H.-S., Cho, M.-C., Lee, H. G., & Yoon, D.-Y. (2010). Indole-3-carbinol induces apoptosis through p53 and activation of caspase-8 pathway in lung cancer A549 cells. *Food and Chemical Toxicology*, *48*(3), 883–890.

Choong, T.-W., He, J., Lee, S. K., & Dodd, I. C. (2016). Growing different lactuca genotypes aeroponically within a tropical greenhouse—cool rootzone temperatures decreased rootzone ethylene concentrations and increased shoot growth. *Frontiers in Physiology*, *7*, 405.

Clarke, S. J., Lamont, K. J., Pan, H. Y., Barry, L. A., Hall, A., & Rogiers, S. Y. (2015). Spring root-zone temperature regulates root growth, nutrient uptake and shoot growth dynamics in grapevines. *Australian Journal of Grape and Wine Research*, *21*(3), 479–489.

Cocetta, G., Mishra, S., Raffaelli, A., & Ferrante, A. (2018). Effect of heat root stress and high salinity on glucosinolates metabolism in wild rocket. *Journal of Plant Physiology*, *231*, 261–270.

Chen, X., C., Zhu, Z., Yang, J, & Liu Y. (2006). Composition and content of glucosinolates in leaves and bolting stems of Chinese kale quantified by HPLC. *Acta Horticulturae Sinica*, *33*(4), 741.

Cooper, A. J. (1973). *Root temperature and plant growth*. Commonwealth Agricultural Bureaux.

Cortés-Olmos, C., Leiva-Brondo, M., Roselló, J., Raigón, M. D., & Cebolla-Cornejo, J. (2014). The role of traditional varieties of tomato as sources of functional compounds. *Journal of the Science of Food and Agriculture*, *94*(14), 2888–2904.

Cumbus, I. P., & Nye, P. H. (1982). Root zone temperature effects on growth and nitrate absorption in rape (*Brassica napus* cv. Emerald). *Journal of Experimental Botany*, *33*(6), 1138–1146.

Cumbus, I. P., & Nye, P. H. (1985). Root zone temperature effects on growth and phosphate absorption in rape (*Brassica napus* cv. Emerald). *Journal of Experimental Botany*, *36*(2), 219–227.

Dalla Costa, L., Tomasi, N., Gottardi, S., Iacuzzo, F., Cortella, G., Manzocco, L., Pinton, R., Mimmo, T., & Cesco, S. (2011). The effect of growth medium temperature on corn salad [*Valerianella locusta* (L.) Laterr] baby leaf yield and quality. *HortScience*, *46*(12), 1619–1625.

Dasika, R., Tangirala, S., & Naishadham, P. (2012). Pesticide residue analysis of fruits and vegetables. *Journal of Environmental Chemistry and Ecotoxicology*, *4*(2), 19–28.

Davidovich-Rikanati, R., Sitrit, Y., Tadmor, Y., Pichersky, E., Dudareva, N., & Lewinsohn, E. (2016). Tomato

aroma: biochemistry and biotechnology. *Biotechnology in Flavor Production*, *39*, 243–263.

De Cnodder, T., Vissenberg, K., Van Der Straeten, D., & Verbelen, J.-P. (2005). Regulation of cell length in the Arabidopsis thaliana root by the ethylene precursor 1-aminocyclopropane-1-carboxylic acid: a matter of apoplastic reactions. *The New Phytologist*, *168*(3), 541–550.

de Koning, A. N. M. (1994). *Development and dry matter distribution in glasshouse tomato : a quantitative approach*. De Koning

Del Carmen Martínez-Ballesta, M., Moreno, D. A., & Carvajal, M. (2013). The physiological importance of glucosinolates on plant response to abiotic stress in Brassica. *International Journal of Molecular Sciences*, *14*(6), 11607–11625.

Del Río-Celestino, M., & Font, R. (2020). The health benefits of fruits and vegetables. *Foods*, *9*(3).

Deng, M., Qian, H., Chen, L., Sun, B., Chang, J., Miao, H., Cai, C., & Wang, Q. (2017). Influence of pre-harvest red light irradiation on main phytochemicals and antioxidant activity of Chinese kale sprouts. *Food Chemistry*, *222*, 1–5.

Díaz-Pérez, J. C. (2009). Root zone temperature, plant growth and yield of broccoli [*Brassica oleracea* (Plenck) var. *italica*] as affected by plastic film mulches. *Scientia Horticulturae*, *123*(2), 156–163.

Díaz-Pérez, J. C., Gitaitis, R., & Mandal, B. (2007). Effects of plastic mulches on root zone temperature and on the manifestation of tomato spotted wilt symptoms and yield of tomato. *Scientia Horticulturae*, *114*(2), 90–95.

Dinkova-Kostova, A. T., & Kostov, R. V. (2012). Glucosinolates and isothiocyanates in health and disease. *Trends in Molecular Medicine*, *18*(6), 337–347.

Dodd, I. C. (2005). Root-to-shoot signalling: assessing the roles of 'up'in the up and down world of long-distance signalling in planta. *Plant and soil*, *274*(1), 251-270.

Dodd, I. C., He, J., Turnbull, C. G., Lee, S. K., & Critchley, C. (2000). The influence of supra-optimal root-zone temperatures on growth and stomatal conductance in *Capsicum annuum* L. *Journal of Experimental Botany*, *51*(343), 239–248.

Doheny-Adams, T., Redeker, K., Kittipol, V., Bancroft, I., & Hartley, S. E. (2017). Development of an efficient glucosinolate extraction method. *Plant Methods*, *13*, 17.

Domínguez, E., Cuartero, J., & Fernández-Muñoz, R. (2005). Breeding tomato for pollen tolerance to low temperatures by gametophytic selection. *Euphytica/ Netherlands Journal of Plant Breeding*, *142*(3), 253–263.

Domisch, T., Finér, L., & Lehto, T. (2001). Effects of soil temperature on biomass and carbohydrate allocation in Scots pine (*Pinus sylvestris*) seedlings at the beginning of the growing season. *Tree Physiology*, *21*(7), 465–472.

Dumas, Y., Dadomo, M., Di Lucca, G., & Grolier, P. (2003). Effects of environmental factors and agricultural techniques on antioxidantcontent of tomatoes. *Journal of the Science of Food and Agriculture*, *83*(5), 369–382.

Engelen-Eigles, G., Holden, G., Cohen, J. D., & Gardner, G. (2006). The effect of temperature, photoperiod, and light quality on gluconasturtiin concentration in watercress (*Nasturtium officinale*R. Br.). *Journal of Agricultural and Food Chemistry*, *54*(2), 328–334.

Engels, C. (1993). Differences between maize and wheat in growth-related nutrient demand and uptake of potassium and phosphorus at suboptimal root zone temperatures. *Plant and Soil*, *150*(1), 129–138.

Engels, C., & Marschner, H. (1992). Root to shoot translocation of macronutrients in relation to shoot demand in maize (*Zea mays* L.) grown at different root zone temperatures. *Zeitschrift fuer Pflanzenernaehrung und Bodenkunde*, *155*(2), 121–128.

Engels, C., Munkle, L., & Marschner, H. (1992). Effect of root zone temperature and shoot demand on uptake and xylem transport of macronutrients in maize (*Zea mays* L.). *Journal of Experimental Botany*, *43*(4), 537–547.

Ensminger, I., Busch, F., & Huner, N. P. A. (2006). Photostasis and cold acclimation: sensing low temperature through photosynthesis. *Physiologia Plantarum*, *126*(1), 28–44.

Equiza, M. (2001). Morphological, anatomical and physiological responses related to differential shoot vs. root growth inhibition at low temperature in spring and winter wheat. *Annals of Botany*, *87*(1), 67–76.

Eremina, M., Rozhon, W., & Poppenberger, B. (2016). Hormonal control of cold stress responses in plants. *Cellular and Molecular Life Sciences*, *73*(4), 797–810.

Erge, H. S., & Karadeniz, F. (2011). Bioactive compounds and antioxidant activity of tomato cultivars. *International Journal of Food Properties*, *14*(5), 968–977.

Fahey, J. W., Wehage, S. L., Holtzclaw, W. D., Kensler, T. W., Egner, P. A., Shapiro, T. A., & Talalay, P. (2012). Protection of humans by plant glucosinolates: efficiency of conversion of glucosinolates to isothiocyanates by the gastrointestinal microflora. *Cancer Prevention Research*, *5*(4), 603–611.

Farnham, M. W., Wilson, P. E., Stephenson, K. K., & Fahey, J. W. (2004). Genetic and environmental effects on glucosinolate content and chemoprotective potency of broccoli. *Plant Breeding*, *123*(1), 60–65.

Feng, H., Yan, M., Fan, X., Li, B., Shen, Q., Miller, A. J., & Xu, G. (2011). Spatial expression and regulation of rice high-affinity nitrate transporters by nitrogen and carbon status. *Journal of Experimental Botany*, *62*(7), 2319–2332.

Fennell, A., & Markhart, A. H. (1998). Rapid acclimation of root hydraulic conductivity to low temperature. *Journal of Experimental Botany*, *49*(322), 879–884.

Fernández-García, E., Carvajal-Lérida, I., Jarén-Galán, M., Garrido-Fernández, J., Pérez-Gálvez, A., & Hornero-Méndez, D. (2012). Carotenoids bioavailability from foods: From plant pigments to efficient biological activities. *Food Research International*, *46*(2), 438–450.

Ferrari, A., Hagedorn, F., & Niklaus, P. A. (2016). Experimental soil warming and cooling alters the partitioning of recent assimilates: evidence from a 14C-labelling study at the alpine treeline. *Oecologia*, *181*(1), 25–37.

Figàs, M. R., Prohens, J., Raigón, M. D., Fita, A., García-Martínez, M. D., Casanova, C., Borràs, D., Plazas, M., Andújar, I., & Soler, S. (2015). Characterization of composition traits related to organoleptic and functional quality for the differentiation, selection and enhancement of local varieties of tomato from different cultivar groups. *Food Chemistry*, 187, 517–524.

Flores, P., Sánchez, E., Fenoll, J., & Hellín, P. (2017). Genotypic variability of carotenoids in traditional tomato cultivars. *Food Research International* , *100*(3), 510–516.

Fuentes, F., Paredes-Gonzalez, X., & Kong, A.-N. T. (2015). Dietary glucosinolates sulforaphane, phenethyl isothiocyanate, indole-3-carbinol/3,3′-diindolylmethane: Antioxidative stress/inflammation, NRF2, epigenetics/epigenomics and in vivo cancer chemopreventive efficacy. *Current Pharmacology Reports*, *1*(3), 179–196.

Fujimura, S., Suzuki, K., Nagao, M., & Okada, M. (2012). Acclimation to root chilling increases sugar concentrations in tomato (*Solanum lycopersicum* L.) fruits. *Scientia Horticulturae*, *147*, 34–41.

Fujishige, N., & Sugiyama, T. (1968). Effect of soil temperature on growth of seedlings of a few fruit vegetables (a preliminary report). *Journal of the Japanese Society for Horticultural Science*, *37*(3), 221–226.

Fujishige, N., Sugiyama, T., & Ogata, R. (1991). Effect of root temperature on the flower formation and fruit yield of tomatoes. *Journal of the Japanese Society for Horticultural Science*, *60*(1), 97–103.

Füllner, K. (2007). *The Influence of Spatially Heterogeneous Soil Temperatures on Plant Structure and Function*. Forschungszentrum Jülich.

Füllner, K., Temperton, V. M., Rascher, U., Jahnke, S., Rist, R., Schurr, U., & Kuhn, A. J. (2012). Vertical

gradient in soil temperature stimulates development and increases biomass accumulation in barley. *Plant, Cell & Environment*, *35*(5), 884–892.

Fu, X., Guo, H., Cong, W., Meng, X.-C., & Others. (2017). Herbal medicine *Radix Scutellariae* quality improved by exposure of the fresh root to high temperature. *Oriental Pharmacy and Experimental Medicine*, *17*(4), 309–313.

Gautier, H., Diakou-Verdin, V., Bénard, C., Reich, M., Buret, M., Bourgaud, F., Poëssel, J. L., Caris-Veyrat, C., & Génard, M. (2008). How does tomato quality (sugar, acid, and nutritional quality) vary with ripening stage, temperature, and irradiance? *Journal of Agricultural and Food Chemistry*, *56*(4), 1241–1250.

Gautier, H., Rocci, A., Buret, M., Grasselly, D., & Causse, M. (2005). Fruit load or fruit position alters response to temperature and subsequently cherry tomato quality. *Journal of the Science of Food and Agriculture*, *85*(6), 1009–1016.

Gazula, A., Kleinhenz, M. D., Streeter, J. G., & Miller, A. R. (2005). Temperature and cultivar effects on anthocyanin and chlorophyll b concentrations in three related Lollo Rosso lettuce cultivars. *HortScience*, *40*(6), 1731–1733.

George, B., Kaur, C., Khurdiya, D. S., & Kapoor, H. C. (2004). Antioxidants in tomato (*Lycopersium esculentum*) as a function of genotype. *Food Chemistry*, *84*(1), 45–51.

George, H. L., Davies, F. S., Crane, J. H., & Schaffer, B. (2002). Root temperature effects on "Arkin" carambola (*Averrhoa carambola* L.) trees: I. Leaf gas exchange and water relations. *Scientia Horticulturae*, *96*(1), 53–65.

Gonzalez-Fuentes, J. A., Shackel, K., Heinrich Lieth, J., Albornoz, F., Benavides-Mendoza, A., & Evans, R. Y. (2016). Diurnal root zone temperature variations affect strawberry water relations, growth, and fruit quality. *Scientia Horticulturae*, *203*, 169–177.

Gosselin, A., & Trudel, M. J. (1982). Influence of substrate temperature on the growth, development and mineral content of tomato plants (cv. Vendor). *Canadian Journal of Plant Science*, *62*: 75l-757.

Gosselin, A., & Trudel, M. J. (1983). Effect of soil heating on plant productivity and energy conservation in northern greenhouses. *III International Symposium on Energy in Protected Cultivation*, *148*, 853–858.

Guerrero-Beltrán, C. E., Calderón-Oliver, M., Pedraza-Chaverri, J., & Chirino, Y. I. (2012). Protective effect of sulforaphane against oxidative stress: recent advances. *Experimental and Toxicologic Pathology*, *64*(5), 503–508.

Gui-xiao, L. A., Ping, F., Ya-juan, L. I., & Yue, W. (2008). Determination of desulpho-glucosinolates in bolting

stems of Chinese kale by liquid chromatography-mass spectrometry [J]. *Journal of Zhejiang University*, *5*.

Guo, J.-J., Tan, X., Fu, H.-L., Chen, J.-X., Lin, X.-X., Ma, Y., & Yang, Z.-Y. (2018). 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. *Journal of Agricultural and Food Chemistry*, *66*(8), 1923–1934.

Guo, L., Yang, R., Wang, Z., Guo, Q., & Gu, Z. (2014). Glucoraphanin, sulforaphane and myrosinase activity in germinating broccoli sprouts as affected by growth temperature and plant organs. *Journal of Functional Foods*, *9*, 70–77.

Guo, R.-F., Yuan, G.-F., & Wang, Q.-M. (2013). Effect of NaCl treatments on glucosinolate metabolism in broccoli sprouts. *Journal of Zhejiang University*, *14*(2), 124–131.

Gupta, P., Wright, S. E., Kim, S.-H., & Srivastava, S. K. (2014). Phenethyl isothiocyanate: a comprehensive review of anti-cancer mechanisms. *Biochimica et Biophysica Acta*, *1846*(2), 405–424.

Guy, C., Kaplan, F., Kopka, J., Selbig, J., & Hincha, D. K. (2008). Metabolomics of temperature stress. *Physiologia Plantarum*, *132*(2), 220–235.

Hamblin, A., Tennant, D., & Perry, M. W. (1990). The cost of stress: Dry matter partitioning changes with seasonal supply of water and nitrogen to dryland wheat. *Plant and Soil*, *122*(1), 47–58.

Hanson, P., Yang, R.-Y., Chang, L.-C., Ledesma, L., & Ledesma, D. (2011). Carotenoids, ascorbic acid, minerals, and total glucosinolates in choysum (*Brassica rapa* cvg. parachinensis) and kailaan (*B. oleraceae Alboglabra* group) as affected by variety and wet and dry season production. *Journal of Food Composition and Analysis*, *24*(7), 950–962.

Hao, H.-P., Jiang, C.-D., Zhang, S.-R., Tang, Y.-D., & Shi, L. (2012). Enhanced thermal-tolerance of photosystem II by elevating root zone temperature in *Prunus mira* Koehne seedlings. *Plant and Soil*, *353*(1-2), 367–378.

Hdider, C., Ilahy, R., Tlili, I., Lenucci, M. S., Dalessandro, G., & Others. (2013). Effect of the stage of maturity on the antioxidant content and antioxidant activity of high-pigment tomato cultivars grown in Italy. *Food*, *7*(1), 1–7.

He, F., Thiele, B., Kraus, D., Bouteyine, S., Watt, M., Kraska, T., Schurr, U., & Kuhn, A. J. (2021). Effects of short-term root cooling before harvest on yield and food quality of Chinese broccoli (*Brassica oleracea* var. *alboglabra* bailey). *Agronomy*, *11*(3), 577.

He, F., Thiele, B., Santhiraraja-Abresch, S., Watt, M., Kraska, T., Ulbrich, A., & Kuhn, A. J. (2020). Effects of

root temperature on the plant growth and food quality of Chinese broccoli (*Brassica oleracea* var. *alboglabra* bailey). *Agronomy*, *10*(5), 702.

He, F., Thiele, B., Watt, M., Kraska, T., Ulbrich, A., & Kuhn, A. J. (2019). Effects of root cooling on plant growth and fruit quality of cocktail tomato during two consecutive seasons. *Journal of Food Quality*, *2019*.

He, J., Qin, L., & Lee, S. K. (2013). Root-zone CO_2 and root-zone temperature effects on photosynthesis and nitrogen metabolism of aeroponically grown lettuce (*Lactuca sativa* L.) in the tropics. *Photosynthetica*, *51*(3), 330–340.

He, J., See, X. E., Qin, L., & Choong, T. W. (2016). Effects of root-zone temperature on photosynthesis, productivity and nutritional quality of aeroponically grown salad rocket (*Eruca sativa*) vegetable. *American Journal of Plant Sciences*, *7*(14), 1993.

He, J., Tan, L. P., & Lee, S. K. (2009). Root-zone temperature effects on photosynthesis, ^{14}C-photoassimilate partitioning and growth of temperate lettuce (*Lactuca sativa* cv. "Panama") in the tropics. *Photosynthetica*, 47(1), 95–103.

Hernández Suárez, M., Rodríguez Rodríguez, E., & Díaz Romero, C. (2008). Analysis of organic acid content in cultivars of tomato harvested in Tenerife. *European Food Research and Technology. A*, *226*(3), 423–435.

He, Y., Yang, J., Zhu, B., & Zhu, Z.-J. (2014). Low root zone temperature exacerbates the ion imbalance and photosynthesis inhibition and induces antioxidant responses in tomato plants under salinity. *Journal of Integrative Agriculture*, *13*(1), 89–99.

Hidaka, K., Yasutake, D., Kitano, M., Takahashi, T., Sago, Y., Ishikawa, K., & Kawano, T. (2007). Production of high quality vegetable by applying low temperature stress to roots. *International Symposium on High Technology for Greenhouse System Management: Greensys2007 801*, 1431–1436.

Hofmann, R. W., & Jahufer, M. Z. Z. (2011). Tradeoff between biomass and flavonoid accumulation in white clover reflects contrasting plant strategies. *PloS One*, *6*(4), e18949.

Huang, X., Lakso, A. N., & Eissenstat, D. M. (2005). Interactive effects of soil temperature and moisture on Concord grape root respiration. *Journal of Experimental Botany*, *56*(420), 2651–2660.

Huot, B., Yao, J., Montgomery, B. L., & He, S. Y. (2014). Growth–defense tradeoffs in plants: a balancing act to optimize fitness. *Molecular Plant*, *7*(8), 1267–1287.

Hurewitz, J., & Janes, H. W. (1983). Effect of altering the root-zone temperature on growth, translocation, carbon exchange rate, and leaf starch accumulation in the tomato. *Plant Physiology*, *73*(1), 46–50.

Hwang, I., Manoharan, R. K., Kang, J.-G., Chung, M.-Y., Kim, Y.-W., & Nou, I.-S. (2016). Genome-wide

identification and characterization of bzip transcription factors in *Brassica oleracea* under cold stress. *BioMed Research International*, *2016*, 4376598.

Ilahy, R., Hdider, C., Lenucci, M. S., Tlili, I., & Dalessandro, G. (2011). Antioxidant activity and bioactive compound changes during fruit ripening of high-lycopene tomato cultivars. *Journal of Food Composition and Analysis*, *24*(4), 588–595.

Ishida, M., Hara, M., Fukino, N., Kakizaki, T., & Morimitsu, Y. (2014). Glucosinolate metabolism, functionality and breeding for the improvement of Brassicaceae vegetables. *Breeding Science*, *64*(1), 48–59.

Islam, M. T. (2011). Effect of temperature on photosynthesis, yield attributes and yield of tomato genotypes. *International Journal of Experimental Agriculture*, *2*(1), 8–11.

Islam, M. Z., Lee, Y.-T., Mele, M. A., Choi, I.-L., & Kang, H.-M. (2019). The effect of phosphorus and root zone temperature on anthocyanin of red romaine lettuce. *Agronomy*, *9*(2), 47.

Ito, A., & Shimizu, H. (2020). Effect of temperature and duration of root chilling on the balance between antioxidant activity and oxidative stress in spinach. *Environmental Control in Biology*, *58*(4), 115–121.

Janská, A., Marsík, P., Zelenková, S., & Ovesná, J. (2010). Cold stress and acclimation - what is important for metabolic adjustment? *Plant Biology*, *12*(3), 395–405.

Jaworska, G. (2005). Content of nitrates, nitrites, and oxalates in New Zealand spinach. *Food Chemistry*, *89*(2), 235–242.

Jia, Y., Wang, J., Qu, Z., Zou, D., Sha, H., Liu, H., Sun, J., Zheng, H., Wang, J., Yang, L., & Zhao, H. (2019). Effects of low water temperature during reproductive growth on photosynthetic production and nitrogen accumulation in rice. *Field Crops Research*, *242*, 107587.

Johkan, M., Ishii, M., Maruo, T., Na, L., Tsukagoshi, S., Hojoh, M.-A., Nakaminami, A., & Shinohara, Y. (2013). Improved light conditions at the fruit truss accelerate harvest time and enhance ascorbic acid concentration in a low-truss, high-density tomato production system. *Journal of the Japanese Society for Horticultural Science*, *82*(4), 317–321.

Justen, V. L., & Fritz, V. A. (2013). Temperature-induced glucosinolate accumulation is associated with expression of BrMYB transcription factors. *HortScience*, *48*(1), 47–52.

Kader, A. A. (1991). Quality and its maintenance in relation to the postharvest physiology of strawberry. *The Strawberry into the 21st Century*. *Timber Press*.

Kader, A. A. (2008). Flavor quality of fruits and vegetables. *Journal of the Science of Food and Agriculture*,

88(11), 1863–1868.

Kalisz, A., Jezdinský, A., Pokluda, R., Sękara, A., Grabowska, A., & Gil, J. (2016). Impacts of chilling on photosynthesis and chlorophyll pigment content in juvenile basil cultivars. *Horticulture, Environment, and Biotechnology*, *57*(4), 330–339.

Kask, K., Kännaste, A., Talts, E., Copolovici, L., & Niinemets, Ü. (2016). How specialized volatiles respond to chronic and short-term physiological and shock heat stress in *Brassica nigra*. *Plant, Cell & Environment*, *39*(9), 2027–2042.

Kato, T., Aoki K., & Yamanishi H. (1995). Effects of low temperatures on the vitamin C, beta-carotene, tocopherol and oxalate contents of spinach grown in the vinyl house. *Japanese Journal of Soil Science and Plant Nutrition*, *66*(5), 563-565.

Kaufmann, M. R. (1975). Leaf water stress in engelmann spruce: influence of the root and shoot environments. *Plant Physiology*, *56*(6), 841–844.

Kawasaki, Y., Matsuo, S., Kanayama, Y., & Kanahama, K. (2014). Effect of root-zone heating on root growth and activity, nutrient uptake, and fruit yield of tomato at low air temperatures. *Journal of the Japanese Society for Horticultural Science*, *83*(4), 295-301.

Kawasaki, Y., Matsuo, S., Suzuki, K., Kanayama, Y., & Kanahama, K. (2013). Root-zone cooling at high air temperatures enhances physiological activities and internal structures of roots in young tomato plants. *Journal of the Japanese Society for Horticultural Science*, *82*(4), 322–327.

Kawasaki, Y., & Yoneda, Y. (2019). Local temperature control in greenhouse vegetable production. *The Horticulture Journal*, *88*(3), 305-314.

Kawashima, L. M., & Valente Soares, L. M. (2003). Mineral profile of raw and cooked leafy vegetables consumed in Southern Brazil. In *Journal of Food Composition and Analysis*, *16*(5), 605–611.

Kawazu, Y., Okimura, M., Ishii, T., & Yui, S. (2003). Varietal and seasonal differences in oxalate content of spinach. *Scientia Horticulturae*, *97*(3), 203–210.

Kayum, M. A., Kim, H.-T., Nath, U. K., Park, J.-I., Kho, K. H., Cho, Y.-G., & Nou, I.-S. (2016). Research on biotic and abiotic stress related genes exploration and prediction in *Brassica rapa* and *B. oleracea*: A review. *Plant Breeding and Biotechnology*, *4*(2), 135–144.

Kelkel, M., Schumacher, M., Dicato, M., & Diederich, M. (2011). Antioxidant and anti-proliferative properties of lycopene. *Free Radical Research*, *45*(8), 925–940.

Kii, S., & Araki, H. (2016). Root-zone cooling improves fruit production in high sugar content tomato. *II Asian*

Horticultural Congress 1208, 291–296.

Kipnis, T., & Dabush, L. (1988). Oxalate accumulation in napier grass and pearl millet× napier grass interspecific hybrids in relation to nitrogen nutrition, irrigation and temperature. *Journal of the Science of Food and Agriculture*, *43*(3), 211–223.

Kissen, R., Eberl, F., Winge, P., Uleberg, E., Martinussen, I., & Bones, A. M. (2016). Effect of growth temperature on glucosinolate profiles in *Arabidopsis thaliana* accessions. *Phytochemistry*, *130*, 106–118.

Kissen, R., Rossiter, J. T., & Bones, A. M. (2009). The "mustard oil bomb": not so easy to assemble?! Localization, expression and distribution of the components of the myrosinase enzyme system. *Phytochemistry Reviews*, *8*(1), 69–86.

Kitano, M, Hidaka K., Zushi K., & Araki T. (2008). Production of value-added vegetables by applying environmental stresses to roots in soil-less culture. *Journal of Science and High Technology in Agriculture*, *20*(4), 204-218.

Kleier, C., Farnsworth, B., & Winner, W. (1998). Biomass, reproductive output, and physiological responses of rapid-cycling Brassica (Brassica rapa) to ozone and modified root temperature. *The New Phytologist*, *139*(4), 657–664.

Koch, K. (2004). Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. *Current Opinion in Plant Biology*, *7*(3), 235–246.

Kong, K.-W., Khoo, H.-E., Prasad, K. N., Ismail, A., Tan, C.-P., & Rajab, N. F. (2010). Revealing the power of the natural red pigment lycopene. *Molecules*, *15*(2), 959–987.

Kopta, T., & Pokluda, R. (2010). Evaluation of organically grown Chinese broccoli (*Brassica oleracea* L. var. alboglabra) under the conditions of the Czech Republic. *XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010)*, *936*, 251–256.

Kotíková, Z., Lachman, J., Hejtmánková, A., & Hejtmánková, K. (2011). Determination of antioxidant activity and antioxidant content in tomato varieties and evaluation of mutual interactions between antioxidants. *LWT - Food Science and Technology*, *44*(8), 1703–1710.

Krumbein, A., Schwarz, D., & Kläring, H.-P. (2012). Effects of environmental factors on carotenoid content in tomato (*Lycopersicon esculentum* (L.) Mill.) grown in a greenhouse. *Journal of Applied Botany and Food Quality*, *80*(2), 160–164.

Kuwagata, T., Ishikawa-Sakurai, J., Hayashi, H., Nagasuga, K., Fukushi, K., Ahamed, A., Takasugi, K., Katsuhara, M., & Murai-Hatano, M. (2012). Influence of low air humidity and low root temperature on

water uptake, growth and aquaporin expression in rice plants. *Plant & Cell Physiology*, *53*(8), 1418–1431.

La, G.-X., Fang, P., Teng, Y.-B., Li, Y.-J., & Lin, X.-Y. (2009). Effect of CO_2 enrichment on the glucosinolate contents under different nitrogen levels in bolting stem of Chinese kale (*Brassica alboglabra* L.). *Journal of Zhejiang University*, *10*(6), 454–464.

La, G.-X., Liu, G.-S., & Fang, P. (2011). Glucosinolates in ten cultivars of Chinese kale (*Brassica alboglabra* L.H Bailey) seperation and identification by HPLC-PAD-ESI/MS. *2011 International Conference on New Technology of Agricultural*, 979-983

Lahti, M., Aphalo, P. J., Finér, L., Ryyppö, A., Lehto, T., & Mannerkoski, H. (2005). Effects of soil temperature on shoot and root growth and nutrient uptake of 5-year-old Norway spruce seedlings. *Tree Physiology*, *25*(1), 115–122.

Lam, V. P., Kim, S. J., Bok, G. J., Lee, J. W., & Park, J. S. (2020). The effects of root temperature on growth, physiology, and accumulation of bioactive compounds of *Agastache rugosa*. *Collection FAO: Agriculture*, *10*(5), 162.

Langi, P., Kiokias, S., Varzakas, T., & Proestos, C. (2018). Carotenoids: From Plants to Food and Feed Industries. *Methods in Molecular Biology* , *1852*, 57–71.

Lee, S. H., Singh, A. P., & Chung, G. C. (2004). Rapid accumulation of hydrogen peroxide in cucumber roots due to exposure to low temperature appears to mediate decreases in water transport. *Journal of Experimental Botany*, *55*(403), 1733–1741.

Lee, S. H., Singh, A. P., Chung, G. C., Ahn, S. J., Noh, E. K., & Steudle, E. (2004). Exposure of roots of cucumber (*Cucumis sativus*) to low temperature severely reduces root pressure, hydraulic conductivity and active transport of nutrients. *Physiologia Plantarum*, *120*(3), 413–420.

Lei, J., Jianjun, L. E. I., Chen, G., Chen, C., & Bihao, C. A. O. (2017). Germplasm Diversity of Chinese Kale in China. *Horticultural Plant Journal*, *3*(3), 101–104.

Leitzmann, C. (1993). Food quality—definition and a holistic view. In *Safeguarding food quality*. Springer, Berlin, Heidelberg.

Leonardi, C., Ambrosino, P., Esposito, F., & Fogliano, V. (2000). Antioxidative activity and carotenoid and tomatine contents in different typologies of fresh consumption tomatoes. *Journal of Agricultural and Food Chemistry*, *48*(10), 4723–4727.

León-Chan, R. G., López-Meyer, M., Osuna-Enciso, T., Sañudo-Barajas, J. A., Heredia, J. B., & León-Félix, J. (2017). Low temperature and ultraviolet-B radiation affect chlorophyll content and induce the

accumulation of UV-B-absorbing and antioxidant compounds in bell pepper (*Capsicum annuum*) plants. *Environmental and Experimental Botany*, *139*, 143–151.

Lewinsohn, E., Sitrit, Y., Bar, E., Azulay, Y., Meir, A., Zamir, D., & Tadmor, Y. (2005). Carotenoid pigmentation affects the volatile composition of tomato and watermelon fruits, as revealed by comparative genetic analyses. *Journal of Agricultural and Food Chemistry*, *53*(8), 3142–3148.

Liu, W., Su, J., Li, S., Lang, X., & Huang, X. (2018). Non-structural carbohydrates regulated by season and species in the subtropical monsoon broad-leaved evergreen forest of Yunnan Province, China. *Scientific Reports*, *8*(1), 1083.

Liu, X., Ren, G., & Shi, Y. (2010). Effect of foliar application steviol glycosides on yield and quality of tomato. *Sugar Tech*, *12*(1), 76–78.

Lloyd, D. T., Soldat, D. J., & Stier, J. C. (2011). Low-temperature nitrogen uptake and use of three cool-season turfgrasses under controlled environments. *HortScience*, *46*(11), 1545–1549.

Lobit, P., Genard, M., Soing, P., & Habib, R. (2006). Modelling malic acid accumulation in fruits: relationships with organic acids, potassium, and temperature. *Journal of Experimental Botany*, *57*(6), 1471–1483.

Camelo, A. F. L. (2004). *Manual for the preparation and sale of fruits and vegetables: from field to market* (Vol. 151). Food & Agriculture Org..

Lunáčková, L., Masarovičová, E., & Lux, A. (2000). Respiration rate and chemical composition of karwinskia roots as affected by temperature. *Biologia Plantarum*, *43*(4), 611–613.

Lu, S., Li, T., Jiang, J., & Others. (2010). Effects of tomato fruit under Na+-salt and Cl--salt stresses on sucrose metabolism. *African Journal of Agricultural Research*, *5*(16), 2227–2231.

Macduff, J. H., Hopper, M. J., & Wild, A. (1987). The effect of root temperature on growth and uptake of ammonium and nitrate by *Brassica napus*L. cv. Bien venu in flowing solution culture. *Journal of Experimental Botany*, *38*(1), 53–66.

Malcolm, P., Holford, P., McGlasson, B., & Barchia, I. (2008). Leaf development, net assimilation and leaf nitrogen concentrations of five Prunus rootstocks in response to root temperature. *Scientia Horticulturae*, *115*(3), 285–291.

Malik, S., Andrade, S. A. L., Sawaya, A. C. H. F., Bottcher, A., & Mazzafera, P. (2013). Root-zone temperature alters alkaloid synthesis and accumulation in *Catharanthus roseus* and *Nicotiana tabacum*. *Industrial Crops and Products*, *49*, 318–325.

Malundo, T. M. M., Shewfelt, R. L., & Scott, J. W. (1995). Flavor quality of fresh tomato (*Lycopersicon*

esculentum Mill.) as affected by sugar and acid levels. *Postharvest Biology and Technology*, *6*(1), 103–110.

Malundo, T. M. M., Shewfelt, R. L., Ware, G. O., & Baldwin, E. A. (2001). Sugars and acids influence flavor properties of mango (*Mangifera indica*). *Journal of the American Society for Horticultural Science*, *126*(1), 115–121.

Marconi, O., Floridi, S., & Montanari, L. (2007). Organic acids profile in tomato juice by HPLC with UV detection. *Journal of Food Quality*, *30*(2), 253-266

Martínez-Valverde, I., Periago, M. J., Provan, G., & Chesson, A. (2002). Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersicum esculentum*). *Journal of the Science of Food and Agriculture*, *82*(3), 323–330.

Massot, C., Bancel, D., Lopez Lauri, F., Truffault, V., Baldet, P., Stevens, R., & Gautier, H. (2013). High temperature inhibits ascorbate recycling and light stimulation of the ascorbate pool in tomato despite increased expression of biosynthesis genes. *PloS One*, *8*(12), e84474.

Massot, C., Génard, M., Stevens, R., & Gautier, H. (2010). Fluctuations in sugar content are not determinant in explaining variations in vitamin C in tomato fruit. *Plant Physiology and Biochemistry*, *48*(9), 751–757.

McMichael, B. L., & Burke, J. J. (1998). Soil temperature and root growth. *HortScience*, *33*.

McMichael, B. L., & Quisenberry, J. E. (1993). The impact of the soil environment on the growth of root systems. *Environmental and Experimental Botany*, *33*(1), 53–61.

Mellidou, I., & Keulemans, J. (2012). Regulation of fruit ascorbic acid concentrations during ripening in high and low vitamin C tomato cultivars. *BMC Plant Biology*, *12*(1), 1-19.

Mithen, R., Bennett, R., & Marquez, J. (2010). Glucosinolate biochemical diversity and innovation in the Brassicales. *Phytochemistry*, *71*(17-18), 2074–2086.

Mohammud, C. H., Illias, M. K., Zaulia, O., Syafk, S. S. A., & Ho, M. Y. A. (2016). Effect of rhizosphere cooling on tomato crop performance under controlled environment structure. *Journal of Tropical Agriculture and Food Science*, *44*(1), 19–27.

Moon, J.-H., Kang, Y. K., & Suh, H.-D. (2006). Effect of root-zone cooling on the growth and yield of cucumber at supraoptimal air temperature. *XXVII International Horticultural Congress-IHC2006: International Symposium on Advances in Environmental Control, Automation 761*, 271–274.

Morison, J. I. L., & Lawlor, D. W. (1999). Interactions between increasing CO_2 concentration and temperature on plant growth. *Plant, Cell & Environment*, *22*(6), 659–682.

Murai-Hatano, M., Kuwagata, T., Sakurai, J., Nonami, H., Ahamed, A., Nagasuga, K., Matsunami, T., Fukushi,

K., Maeshima, M., & Okada, M. (2008). Effect of low root temperature on hydraulic conductivity of rice plants and the possible role of aquaporins. *Plant and Cell Physiology*, *49*(9), 1294–1305.

Nagasuga, K., Murai-Hatano, M., & Kuwagata, T. (2011). Effects of low root temperature on dry matter production and root water uptake in rice plants. *Plant Production Science*, *14*(1), 22–29.

Nagel, K. A., Kastenholz, B., Jahnke, S., van Dusschoten, D., Aach, T., Mühlich, M., Truhn, D., Scharr, H., Terjung, S., Walter, A., & Schurr, U. (2009). Temperature responses of roots: impact on growth, root system architecture and implications for phenotyping. *Functional Plant Biology: FPB*, *36*(11), 947–959.

Nguyen, D. T. P., Lu, N., Kagawa, N., Kitayama, M., & Takagaki, M. (2020). Short-term root-zone temperature treatment enhanced the accumulation of secondary metabolites of hydroponic coriander (*Coriandrum sativum* L.) grown in a plant factory. *Agronomy*, *10*(3), 413.

Nguyen, Nguyen, Lu, Kagawa, & Takagaki. (2019). Optimization of photosynthetic photon flux density and root-zone temperature for enhancing secondary metabolite accumulation and production of coriander in plant factory. *Agronomy*, *9*(5), 224.

Niam, A. G., & Suhardiyanto, H.. Root-zone cooling in tropical greenhouse: a review. *IOPscience*, *557*(1), 012144.

Nkansah, G. O., & Ito, T. (1995). Effect of air and root-zone temperatures on physiological characteristics and yield of heat-tolerant and non heat-tolerant tomato cultivars. *Journal of the Japanese Society for Horticultural Science*, *64*(2), 315–320.

Ntatsi, G., Savvas, D., Huntenburg, K., Druege, U., Hincha, D. K., Zuther, E., & Schwarz, D. (2014). A study on ABA involvement in the response of tomato to suboptimal root temperature using reciprocal grafts with notabilis, a null mutant in the ABA-biosynthesis gene LeNCED1. *Environmental and Experimental Botany*, 97, 11–21.

Ntatsi, G., Savvas, D., Kläring, H.-P., & Schwarz, D. (2014). Growth, yield, and metabolic responses of temperature-stressed tomato to grafting onto rootstocks differing in cold tolerance. *Journal of the American Society for Horticultural Science*, *139*(2), 230–243.

Ntatsi, G., Savvas, D., Papasotiropoulos, V., Katsileros, A., Zrenner, R. M., Hincha, D. K., Zuther, E., & Schwarz, D. (2017). Rootstock sub-optimal temperature tolerance determines transcriptomic responses after long-term root cooling in rootstocks and scions of grafted tomato plants. *Frontiers in Plant Science*, *8*, 911.

Oerlemans, K., Barrett, D. M., Suades, C. B., Verkerk, R., & Dekker, M. (2006). Thermal degradation of

glucosinolates in red cabbage. *Food Chemistry*, *95*(1), 19–29.

Ogawa, E., Hikosaka, S., & Goto, E. (2018). Effects of nutrient solution temperature on the concentration of major bioactive compounds in red perilla. *Journal of Agricultural Meteorology*, *74*(2), 71–78.

Okuda, N., Fujime, Y., & Varanyanond, W. (1997). Earliness and self-compatibility of Chinese kale (*Brassica oleracea* L. var. alboglabra LH Bailey). *Acta Horticulturae, 533*(533):89-100.

Olberg, M. W., & Lopez, R. G. (2016). Growth and development of poinsettia (*Euphorbia pulcherrima*) finished under reduced air temperature and bench-top root-zone heating. *Scientia Horticulturae, 210*, 197–204.

Olberg, M. W., & Lopez, R. G. (2017). Bench-top root-zone heating hastens development of petunia under a lower air temperature. *HortScience*, *52*(1), 54–59.

Olle, M., & Bender, I. (2009). Causes and control of calcium deficiency disorders in vegetables: a review. *The Journal of Horticultural Science & Biotechnology*, *84*(6), 577–584.

Padilla, G., Cartea, M. E., Velasco, P., de Haro, A., & Ordás, A. (2007). Variation of glucosinolates in vegetable crops of *Brassica rapa*. *Phytochemistry*, *68*(4), 536–545.

Panthee, D. R., Labate, J. A., McGrath, M. T., Breksa, A. P., & Robertson, L. D. (2013). Genotype and environmental interaction for fruit quality traits in vintage tomato varieties. *Euphytica/ Netherlands Journal of Plant Breeding*, *193*(2), 169–182.

Patrick, J. W., Botha, F. C., & Birch, R. G. (2013). Metabolic engineering of sugars and simple sugar derivatives in plants. *Plant Biotechnology Journal*, *11*(2), 142–156.

Paul, M. J., & Foyer, C. H. (2001). Sink regulation of photosynthesis. *Journal of Experimental Botany*, *52*(360), 1383–1400.

Pereira, F. M. V., Rosa, E., Fahey, J. W., Stephenson, K. K., Carvalho, R., & Aires, A. (2002). Influence of temperature and ontogeny on the levels of glucosinolates in broccoli (*Brassica oleracea* Var.*italica*) sprouts and their effect on the induction of mammalian phase 2 enzymes. *Journal of Agricultural and Food Chemistry*, *50*(21), 6239–6244.

Perera, C. O., & Yen, G. M. (2007). Functional properties of carotenoids in human health. *International Journal of Food Properties*, *10*(2), 201–230.

Pettersson, S. (1995). Low root zone temperature effects on net mineral nutrient uptake and distribution in barley (*Hordeum vulgare*). *Journal of Plant Physiology*, *145*(4), 459–464.

Peuke, A. D., Windt, C., & Van As, H. (2006). Effects of cold-girdling on flows in the transport phloem in

Ricinus communis: is mass flow inhibited? *Plant, Cell and Environment*, *29*(1), 15–25.

Poiré, R., Schneider, H., Thorpe, M. R., Kuhn, A. J., Schurr, U., & Walter, A. (2010). Root cooling strongly affects diel leaf growth dynamics, water and carbohydrate relations in Ricinus communis. *Plant, Cell & Environment*, *33*(3), 408–417.

Poiroux-Gonord, F., Bidel, L. P. R., Fanciullino, A.-L., Gautier, H., Lauri-Lopez, F., & Urban, L. (2010). Health benefits of vitamins and secondary metabolites of fruits and vegetables and prospects to increase their concentrations by agronomic approaches. *Journal of Agricultural and Food Chemistry*, *58*(23), 12065–12082.

Pollock, C. J., & Eagles, C. F. (1988). Low temperature and the growth of plants. *Symposia of the Society for Experimental Biology*, *42*, 157–180.

Qian, H., Liu, T., Deng, M., Miao, H., Cai, C., Shen, W., & Wang, Q. (2016). Effects of light quality on main health-promoting compounds and antioxidant capacity of Chinese kale sprouts. *Food Chemistry*, *196*, 1232–1238.

Quadir, Q. F., Watanabe, T., Chen, Z., Osaki, M., & Shinano, T. (2011). Ionomic response of Lotus japonicus to different root-zone temperatures. *Soil Science and Plant Nutrition*, *57*(2), 221–232.

Raffo, A., Salucci, M., AzzinI, E., Bertone, A., Quaglia, G. B., Fogliano, V., Graziani, G., & La Malfa, G. (2002). Nutritional characteristics of greenhouse cherry tomatoes. *VI International Symposium on Protected Cultivation in Mild Winter Climate: Product and Process Innovation*, *614*, 681–686.

Raiola, A., Rigano, M. M., Calafiore, R., Frusciante, L., & Barone, A. (2014). Enhancing the health-promoting effects of tomato fruit for biofortified food. *Mediators of Inflammation*, *2014*, 139873.

Ramakrishna, A., & Ravishankar, G. A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling & Behavior*, *6*(11), 1720–1731.

Ripoll, J., Urban, L., Brunel, B., & Bertin, N. (2016). Water deficit effects on tomato quality depend on fruit developmental stage and genotype. *Journal of Plant Physiology*, *190*, 26–35.

Ripoll, J., Urban, L., Staudt, M., Lopez-Lauri, F., Bidel, L. P. R., & Bertin, N. (2014). Water shortage and quality of fleshy fruits—making the most of the unavoidable. *Journal of Experimental Botany*, *65*(15), 4097–4117.

Rosa, E. A. S., & Rodrigues, P. M. F. (1998). The effect of light and temperature on glucosinolate concentration in the leaves and roots of cabbage seedlings. *Journal of the Science of Food and Agriculture*, *78*(2), 208–212.

Rosa, E., David, M., & Gomes, M. H. (2001). Glucose, fructose and sucrose content in broccoli, white cabbage and Portuguese cabbage grown in early and late seasons. *Journal of the Science of Food and Agriculture*, *81*(12), 1145–1149.

Rosales, M. A., Ruiz, J. M., Hernández, J., Soriano, T., Castilla, N., & Romero, L. (2006). Antioxidant content and ascorbate metabolism in cherry tomato exocarp in relation to temperature and solar radiation. *Journal of the Science of Food and Agriculture*, *86*(10), 1545–1551.

Roselló, S., Adalid, A. M., Cebolla-Cornejo, J., & Nuez, F. (2011). Evaluation of the genotype, environment and their interaction on carotenoid and ascorbic acid accumulation in tomato germplasm. *Journal of the Science of Food and Agriculture*, *91*(6), 1014–1021.

Ruelland, E., Vaultier, M.-N., Zachowski, A., & Hurry, V. (2009). Chapter 2 cold signalling and cold acclimation in plants. *Advances in Botanical Research*, 49, 35–150.

Rylski, I. (1979). Fruit set and development of seeded and seedless tomato fruits under diverse regimes of temperature and pollination. *Journal of the American Society for Horticultural Science*, *104*.

Sago, Y., Yasutake, D., Hidaka, K., Yasunaga, E., Eguchi, T., Yoshida, S., & Kitano, M. (2011a). Kinetics of root ion absorption affected by environmental factors and transpiration ii. Environmental effects and a concentration-dependent model. *Environmental Control in Biology*, *49*(1), 33–40.

Sago, Y., Yasutake, D., Hidaka, K., Yasunaga, E., Eguchi, T., Yoshida, S., & Kitano, M. (2011b). Kinetics of root ion absorption affected by environmental factors and transpiration i. Measurement system for intact roots. *Environmental Control in Biology*, *49*(1), 23–31.

Sakamoto, M., & Suzuki, T. (2015a). Effect of root-zone temperature on growth and quality of hydroponically grown red leaf lettuce (*Lactuca sativa* L. cv. Red Wave). *American Journal of Plant Sciences*, *6*(14), 2350.

Sakamoto, M., & Suzuki, T. (2015b). Elevated root-zone temperature modulates growth and quality of hydroponically grown carrots. *Agricultural Sciences in China*, *6*(08), 749.

Sakamoto, M., Uenishi, M., Miyamoto, K., & Suzuki, T. (2016). Effect of root-zone temperature on the growth and fruit quality of hydroponically grown strawberry plants. *The Journal of Agricultural Science*, *8*, 122–131.

Salehi, B., Sharifi-Rad, R., Sharopov, F., Namiesnik, J., Roointan, A., Kamle, M., Kumar, P., Martins, N., & Sharifi-Rad, J. (2019). Beneficial effects and potential risks of tomato consumption for human health: An overview. *Nutrition* , *62*, 201–208.

Sami, F., Yusuf, M., Faizan, M., Faraz, A., & Hayat, S. (2016). Role of sugars under abiotic stress. *Plant*

Physiology and Biochemistry, *109*, 54–61.

Sawhney, V. K., & Polowick, P. L. (1985). Fruit development in tomato: the role of temperature. *Canadian Journal of Botany*, *63*(6), 1031–1034.

Schulz, E., Tohge, T., Zuther, E., Fernie, A. R., & Hincha, D. K. (2016). Flavonoids are determinants of freezing tolerance and cold acclimation in *Arabidopsis thaliana*. *Scientific Reports*, *6*, 34027.

Schwarz, D., Rouphael, Y., Colla, G., & Venema, J. H. (2010). Grafting as a tool to improve tolerance of vegetables to abiotic stresses: Thermal stress, water stress and organic pollutants. *Scientia Horticulturae*, *127*(2), 162–171.

Selli, S., Kelebek, H., Ayseli, M. T., & Tokbas, H. (2014). Characterization of the most aroma-active compounds in cherry tomato by application of the aroma extract dilution analysis. *Food Chemistry*, *165*, 540–546.

Setter, T. L., & Greenway, H. (1988). Growth reductions of rice at low root temperature: decreases in nutrient uptake and development of chlorosis. *Journal of Experimental Botany*, *39*(6), 811–829.

Sharma, P., Jha, A. B., Dubey, R. S., & Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany*, *2012*, 1–26.

Shaw, D. V. (1990). Response to selection and associated changes in genetic variance for soluble solids and titratable acids contents in strawberries. *Journal of the American Society for Horticultural Science*, *115*(5), 839–843.

Shimono, H., Hasegawa, T., Fujimura, S., & Iwama, K. (2004). Responses of leaf photosynthesis and plant water status in rice to low water temperature at different growth stages. *Field Crops Research*, *89*(1), 71–83.

Siddiqui, M. W., Ayala-Zavala, J. F., & Dhua, R. S. (2015). Genotypic variation in tomatoes affecting processing and antioxidant attributes. *Critical Reviews in Food Science and Nutrition*, *55*(13), 1819–1835.

Singh, K., Connors, S. L., Macklin, E. A., Smith, K. D., Fahey, J. W., Talalay, P., & Zimmerman, A. W. (2014). Sulforaphane treatment of autism spectrum disorder (ASD). *Proceedings of the National Academy of Sciences of the United States of America*, *111*(43), 15550–15555.

Slavin, J. L., & Lloyd, B. (2012). Health benefits of fruits and vegetables. *Advances in Nutrition* , *3*(4), 506–516.

Solfjeld, I., & Johnsen, Ø. (2006). The influence of root-zone temperature on growth of *Betula pendula* Roth. *Trees*, *20*(3), 320–328.

Sønderby, I. E., Geu-Flores, F., & Halkier, B. A. (2010). Biosynthesis of glucosinolates – gene discovery and beyond. *Trends in Plant Science*, *15*(5), 283–290.

Sonntag, F., Naumann, M., Pawelzik, E., & Smit, I. (2019). Improvement of cocktail tomato yield and consumer-oriented quality traits by potassium fertilization is driven by the cultivar. *Journal of the Science of Food and Agriculture*, *99*(7), 3350–3358.

Soydam Aydin, S., Büyük, I., & Aras, S. (2013). Relationships among lipid peroxidation, SOD enzyme activity, and SOD gene expression profile in *Lycopersicum esculentum* L. exposed to cold stress. *Genetics and Molecular Research: GMR*, *12*(3), 3220–3229.

Starck, Z., Niemyska, B., Bogdan, J., & Tawalbeh, R. N. A. (2000). Response of tomato plants to chilling stress in association with nutrient or phosphorus starvation. *Plant and Soil*, *226*(1), 99–106.

Steindal, A. L. H., Mølmann, J., Bengtsson, G. B., & Johansen, T. J. (2013). Influence of day length and temperature on the content of health-related compounds in broccoli (*Brassica oleracea* L. var. italica). *Journal of Agricultural and Food Chemistry*, *61*(45), 10779–10786.

Steindal, A. L. H., Rødven, R., Hansen, E., & Mølmann, J. (2015). Effects of photoperiod, growth temperature and cold acclimatisation on glucosinolates, sugars and fatty acids in kale. *Food Chemistry*, *174*, 44–51.

Stewart, A. J., Bozonnet, S., Mullen, W., Jenkins, G. I., Lean, M. E., & Crozier, A. (2000). Occurrence of flavonols in tomatoes and tomato-based products. *Journal of Agricultural and Food Chemistry*, *48*(7), 2663–2669.

Story, E. N., Kopec, R. E., Schwartz, S. J., & Harris, G. K. (2010). An update on the health effects of tomato lycopene. *Annual Review of Food Science and Technology*, *1*, 189–210.

Sun, B., Liu, N., Zhao, Y., Yan, H., & Wang, Q. (2011). Variation of glucosinolates in three edible parts of Chinese kale (*Brassica alboglabra* Bailey) varieties. *Food Chemistry*, *124*(3), 941–947.

Sun, B., Yan, H., Liu, N., Wei, J., & Wang, Q. (2012a). Effect of 1-MCP treatment on postharvest quality characters, antioxidants and glucosinolates of Chinese kale. *Food Chemistry*, *131*(2), 519–526.

Sun, B., Yan, H., Zhang, F., & Wang, Q. (2012b). Effects of plant hormones on main health-promoting compounds and antioxidant capacity of Chinese kale. *Food Research International* , *48*(2), 359–366.

Sun, J., Lu, N., Xu, H., Maruo, T., & Guo, S. (2016). Root zone cooling and exogenous spermidine root-pretreatment promoting *Lactuca sativa* L. Growth and photosynthesis in the high-temperature season. *Frontiers in Plant Science*, *7*, 368.

Suzuki, K., Nagasuga, K., & Okada, M. (2008). The chilling injury induced by high root temperature in the

leaves of rice seedlings. *Plant and Cell Physiology*, *49*(3), 433–442.

Tan, L. P., He, J., & Lee, S. K. (2002). Effects of root-zone temperature on the root development and nutrient uptake of *Lactuca sativa* L.“Panama” grown in an aeroponic system in the tropics. *Journal of Plant Nutrition*, *25*(2), 297-314.

Taranet, P., Kirchhof, G., Fujinuma, R., & Menzies, N. (2018). Root zone temperature alters storage root formation and growth of sweetpotato. *Journal of Agronomy and Crop Science*, *204*(3), 313–324.

Thompson, K. A., Marshall, M. R., Sims, C. A., Wei, C. I., Sargent, S. A., & Scott, J. W. (2000). Cultivar, maturity, and heat treatment on lycopene content in tomatoes. *Journal of Food Science*, *65*(5), 791–795.

Tindall, J. A., Mills, H. A., & Radcliffe, D. E. (1990). The effect of root zone temperature on nutrient uptake of tomato. *Journal of Plant Nutrition*, *13*(8), 939–956.

Toor, R. K., Savage, G. P., & Lister, C. E. (2006). Seasonal variations in the antioxidant composition of greenhouse grown tomatoes. *Journal of Food Composition and Analysis*, *19*(1), 1–10.

Tronstad, R. (1995). Importance of melon type, size, grade, container, and season in determining melon prices. *Journal of Agricultural and Resource Economics*, *20*(1), 32–48.

Trudel, M. J., Mj, T.. (1982). Influence of soil temperature in greenhouse tomato production. *HortScience*, *17*(6), 928-929

Valtueña, J., Breidenassel, C., Folle, J., & Gonzalez-Gross, M. (2011). Retinol, B-carotene, a-tocopherol and vitamin D status in European adolescents; regional differences and variability: A review. *Nutricion Hospitalaria*, *26*(2), 280–288.

Van Ploeg, D., & Heuvelink, E. (2005). Influence of sub-optimal temperature on tomato growth and yield: a review. *The Journal of Horticultural Science & Biotechnology*, *80*(6), 652–659.

Variyar, P. S., Banerjee, A., Akkarakaran, J. J., & Suprasanna, P. (2014). Role of glucosinolates in plant stress tolerance. *Emerging Technologies and Management of Crop Stress Tolerance*. Academic Press.

Venema, J. H., Dijk, B. E., Bax, J. M., van Hasselt, P. R., & Elzenga, J. T. M. (2008). Grafting tomato (*Solanum lycopersicum*) onto the rootstock of a high-altitude accession of *Solanum habrochaites* improves suboptimal-temperature tolerance. *Environmental and Experimental Botany*, *63*(1), 359–367.

Veselova, S. V., Farhutdinov, R. G., Veselov, S. Y., Kudoyarova, G. R., Veselov, D. S., & Hartung, W. (2005). The effect of root cooling on hormone content, leaf conductance and root hydraulic conductivity of durum wheat seedlings (*Triticum durum* L.). *Journal of Plant Physiology*, *162*(1), 21–26.

Vincente, A. R., Manganaris, G. A., Ortiz, C. M., Sozzi, G. O., & Crisosto, C. H. (2014). Chapter 5 - Nutritional

quality of fruits and vegetables, *Postharvest Handling (Third Edition)*. Academic Press.

Viola, R., & Davies, H. V. (1992). A microplate reader assay for rapid enzymatic quantification of sugars in potato tubers. *Potato Research*, *35*(1), 55–58.

Viuda-Martos, M., Sanchez-Zapata, E., Sayas-Barberá, E., Sendra, E., Pérez-Álvarez, J. A., & Fernández-López, J. (2014). Tomato and tomato byproducts. Human health benefits of lycopene and its application to meat products: a review. *Critical Reviews in Food Science and Nutrition*, *54*(8), 1032–1049.

Volden, J., Bengtsson, G. B., & Wicklund, T. (2009). Glucosinolates, l-ascorbic acid, total phenols, anthocyanins, antioxidant capacities and colour in cauliflower (*Brassica oleracea* L. ssp. botrytis); effects of long-term freezer storage. *Food Chemistry*, *112*(4), 967–976.

Wang, H., Wu, J., Sun, S., Liu, B., Cheng, F., Sun, R., & Wang, X. (2011). Glucosinolate biosynthetic genes in Brassica rapa. *Gene*, *487*(2), 135–142.

Wang, L.-J., Huang, W.-D., Li, J.-Y., Liu, Y.-F., & Shi, Y.-L. (2004). Peroxidation of membrane lipid and Ca^{2+} homeostasis in grape mesophyll cells during the process of cross-adaptation to temperature stresses. *Plant Science*, *167*(1), 71–77.

Wang, S. Y., & Camp, M. J. (2000). Temperatures after bloom affect plant growth and fruit quality of strawberry. *Scientia Horticulturae*, *85*(3), 183–199.

Wang, X., Zhang, W., Miao, Y., & Gao, L. (2016). Root-zone warming differently benefits mature and newly unfolded leaves of *cucumis sativus* l. seedlings under sub-optimal temperature stress. *PloS One*, *11*(5), e0155298.

Wang, Y.-Q., Hu, L.-P., Liu, G.-M., Zhang, D.-S., & He, H.-J. (2017). Evaluation of the nutritional quality of Chinese kale (*Brassica alboglabra* Bailey) using UHPLC-Quadrupole-Orbitrap MS/MS-based metabolomics. *Molecules* , *22*(8).

Wang, Y., Roger Illingworth, D., Connor, S. L., Barton Duell, P., & Connor, W. E. (2010). Competitive inhibition of carotenoid transport and tissue concentrations by high dose supplements of lutein, zeaxanthin and beta-carotene. *European Journal of Nutrition*, *49*(6), 327–336.

Wan, X., Landhäusser, S. M., Zwiazek, J. J., & Lieffers, V. J. (1999). Root water flow and growth of aspen (*Populus tremuloides*) at low root temperatures. *Tree Physiology*, *19*(13), 879–884.

Wan, X., Zwiazek, J. J., Lieffers, V. J., & Landhäusser, S. M. (2001). Hydraulic conductance in aspen (*Populus tremuloides*) seedlings exposed to low root temperatures. *Tree Physiology*, *21*(10), 691–696.

White, P. J., Clarkson, D. T., & Earnshaw, M. J. (1987). Acclimation of potassium influx in rye (*Secale cereale*)

to low root temperatures. *Planta*, *171*(3), 377–385.

Wieczorek, M. N., Walczak, M., Skrzypczak-Zielińska, M., & Jeleń, H. H. (2018). Bitter taste of Brassica vegetables: The role of genetic factors, receptors, isothiocyanates, glucosinolates, and flavor context. *Critical Reviews in Food Science and Nutrition*, *58*(18), 3130–3140.

Wilson, J. B. (1988). A Review of evidence on the control of shoot: root ratio, in relation to models. *Annals of Botany*, *61*(4), 433–449.

Wu, M., & Kubota, C. (2008). Effects of high electrical conductivity of nutrient solution and its application timing on lycopene, chlorophyll and sugar concentrations of hydroponic tomatoes during ripening. *Scientia Horticulturae*, *116*(2), 122–129.

Xian, Y., & Yunying, Y. (2002). The effects of temperature on flower bud differentiation, yield and quality formation in chinese kale (*Brassica alboglabra* Bailey). *Journal of South China Agricultural University*, *23*(2), 5–7.

Yang, X., & Yang, Y. (2002). The effects of temperature on flower bud differentiation, yield and quality formation in Chinese kale (*Brassica alboglabra* Bailey). *Journal of South China Agricultural University*, *23*(2), 5–7.

Yang, Y.-W., Chen, H.-C., Jen, W.-F., Liu, L.-Y., & Chang, M.-C. (2015). Comparative transcriptome analysis of shoots and roots of TNG67 and TCN1 rice seedlings under cold stress and following subsequent recovery: Insights into metabolic pathways, phytohormones, and transcription factors. *PLOS ONE*, *10*(7), e0131391.

Yan, Q., Duan, Z., Mao, J., Li, X., & Dong, F. (2012a). Effects of root-zone temperature and N, P, and K supplies on nutrient uptake of cucumber (*Cucumis sativus* L.) seedlings in hydroponics. *Soil Science and Plant Nutrition*, *58*(6), 707–717.

Yan, Q.-Y., Duan, Z.-Q., Mao, J.-D., Li, X., & Dong, F. (2013). Low root zone temperature limits nutrient effects on cucumber seedling growth and induces adversity physiological response. *Journal of Integrative Agriculture*, *12*(8), 1450–1460.

Yin, L., Chen, C., Chen, G., Cao, B., & Lei, J. (2015). Molecular cloning, expression pattern and genotypic effects on glucoraphanin biosynthetic related genes in Chinese kale (*Brassica oleracea* var. *alboglabra* Bailey). *Molecules*, *20*(11), 20254–20267.

Yuanyuan, M., Yali, Z., Jiang, L., & Hongbo, S. (2009). Roles of plant soluble sugars and their responses to plant cold stress. *African Journal of Biotechnology*, *8*(10).

Zanfini, A., Franchi, G. G., Massarelli, P., Corbini, G., & Dreassi, E. (2017). Phenolic compounds , carotenoids and antioxidant activity in five tomato (*Lycopesicon esculentum* mill.) cultivars. *Italian Journal of Food Science*, *29*(1).

Zhang, S., & Dang, Q.-L. (2007). Interactive effects of soil temperature and [CO_2] on morphological and biomass traits in seedlings of four boreal tree species. *Forest Science*, *53*(3), 453–460.

Zhang, Y.-P., Jia, F.-F., Zhang, X.-M., Qiao, Y.-X., Shi, K., Zhou, Y.-H., & Yu, J.-Q. (2012). Temperature effects on the reactive oxygen species formation and antioxidant defence in roots of two cucurbit species with contrasting root zone temperature optima. *Acta Physiologiae Plantarum*, *34*(2), 713–720.

Zhang, Y. P., Qiao, Y. X., Zhang, Y. L., Zhou, Y. H., & Yu, J. Q. (2008). Effects of root temperature on leaf gas exchange and xylem sap abscisic acid concentrations in six Cucurbitaceae species. *Photosynthetica*, *46*(3), 356.

Zhang, Y., Zhang, Y., Zhou, Y., & Yu, J. (2007). Adaptation of cucurbit species to changes in substrate temperature: Root growth, antioxidants, and peroxidation. *Journal of Plant Biology*, *50*(5), 527–532.