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Effects of Root Temperature and Cluster Position on Fruit Quality of Two Cocktail Tomato Cultivars

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Abstract: Managing root temperature can strongly influence plant growth in various species. Our previous work has shown that a positive effect of root cooling on the nutritional quality (sugars, vitamin C, and carotenoids levels) of two cocktail tomato cultivars, while the yield and mineral content was moderately reduced in two seasons. But few studies have investigated which cluster quality is more influenced by root temperature. The main purpose of this study was to evaluate the effects of root temperature and different cluster positions (the first to the fourth clusters) on fruit quality parameters of two cocktail tomato cultivars ('Amoroso' and 'Delioso'). Two root temperatures, cool (10 °C) and control (18–22 °C), were applied to the roots of hydroponically cultivated tomato plants after inflorescence until the final harvest in the greenhouse. The results showed that root cooling has no influence on the biomass of fruits (both dry weight and fresh weight) of all clusters, but it increased the sugar (6.1–8.4%) and vitamin C (9.1–12.5%) concentration of the second cluster of 'Amoroso' and the third cluster of 'Delioso' compared to the control. In most cases, significant positive changes (8–23.8%) in the levels of lycopene were observed in response to low root temperature treatment, but the effect was dependent on different cluster positions and different cultivars. Most mineral element levels showed no response to the root temperature. Significant reductions (10% and 17.6%) along with the root cooling was only found in the level of zinc and potassium of the first and the second cluster of 'Delioso', respectively. It was concluded that the fruits from the second or third clusters containing more sugars, vitamin C, and lycopene, were more influenced by root cooling. In addition, the effect of cluster position on fruit quality was different between two root temperature groups. Root cooling reduced the heterogeneity in fruit quality parameters among the clusters of 'Amoroso' and increased the differences in 'Delioso'.

Keywords: cocktail tomato; hydroponic; antioxidants; sugar; lycopene; ascorbic acids

Citation: He, F.; Thiele, B.; Kraska, T.; Schurr, U.; Kuhn, A.J. Effects of Root Temperature and Cluster Position on Fruit Quality of Two Cocktail Tomato Cultivars. *Agronomy* **2022**, *12*, 1275. https://doi.org/10.3390/agronomy12061275

Academic Editor: Luis Noguera-Artiaga

Received: 28 April 2022
Accepted: 23 May 2022
Published: 26 May 2022

1. Introduction

Tomato (*Solanum lycopersicum*), one of the most cultivated economical crops around the world (FAO, 2020), has long been recognized as a healthy vegetable or fruit due to its high concentration of antioxidants and minerals, such as carotenoids, vitamin C, and potassium [1]. Among various nutrients in tomatoes, lycopene is the most prominent antioxidant and is also the most effective free-radical scavenger in vitro of all carotenoids [2]. Numerous studies have proven that lycopene plays a beneficial role in chronic diseases [3]. Tomato provides an important dietary source of vitamin C (ascorbic acid) due to its widespread consumption [4]. In addition, tomatoes are a good source of essential minerals for humans, such as Fe and K [5].

Root temperature is one of the environmental factors that regulates the growth, biomass distribution, and food composition of horticultural crops [6–8]. Changing the

root temperature often influences the concentrations of some primary and most secondary metabolites [9]. Basically, root temperature alteration during plant growth can cause oxidative stress by enhanced formation and accumulation of reactive oxygen species (ROS). Thus, the synthesis of low-molecular-weight compounds are provoked to counteract the oxidative damage caused by ROS [10]. Most of these compounds are antioxidants, or soluble sugars, which are desired nutrients in fruits or vegetables. Therefore, applying root temperature is an effective method to improve the food quality of horticultural crops considering the nutritional composition. For example, in *Agastache rugosa*, reducing the root temperature to 10 °C for 24-day has increased the concentration of rosmarinic acid and acacetin [11]. Similarly, reducing the fertigation temperature at 4 °C to 5 °C during the growth of saffron plants has been found to have a positive impact on the flower quality regarding the increased safranal and crocin concentration [12]. Moreover, another advantage of root temperature management is its relative convenience and energy efficiency compared to air temperature controlling [13].

Fruit position has been reported to influence the maturity, and assimilates transport and fruit quality of different horticultural crops as well [14,15]. The concentration of secondary metabolites, including anthocyanin, flavonoids, and phenolic compounds, differed at different positions on the truss of strawberries, but the firmness was not influenced [16,17]. Fruit position on the shoot of peach plant showed a significant influence on peach fruit firmness and color, so the distal fruits were smaller, but presented lower firmness, and lighter skin [16]. In addition, Pek et al. [18] found that tomato fruits from non-shaded positions contained less lycopene, but more polyphenols and ascorbic acid content than fruits from shaded positions. These differences could be due to assimilated competition, exposure to sunlight, and orientation [19–21]. Our previous study has found that root cooling has some beneficial effect on fruit composition (sugars, vitamin C, and carotenoids) and a modest negative effect on the yield and mineral elements of two cocktail tomato cultivars ('Amoroso' and 'Delioso') in winter and summer [22]. However, how root cooling based on cluster position influences commercial and functional tomato quality is unknown.

In this regard, the main aim of the study was to determine the effect of root-cooling treatment and the cluster position (first to the fourth cluster) on fruit quality parameters (weight, diameter, and soluble solid content), as well as in sugar, carotenoids, organic acids and mineral levels. For this purpose, the same two cocktail tomato cultivars ('Amoroso' and 'Delioso') as our previous studies were considered. Root-cooling treatment was constructed in the generative stage to minimize the negative effect on production and yield [8].

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Cocktail tomato (*Solanum lycopersicon*, cv 'Amoroso' and cv 'Delioso', Rijk Zwaan, The Netherlands) was cultivated hydroponically with a single-truss high-wire system in the greenhouse in Forschungszentrum Jülich, Germany, from October 2018 to April 2019. Seeds were sown into Rockwool plugs (25 × 25 × 40 mm with a 6/16 mm hole, Grodan Vital, Roermond, The Netherlands) and kept in darkness at 25 °C. After the 1st true leaf was developed at 15 DAS (days after sowing), seedlings were transferred to Rockwool cubes (100 × 100 × 65 mm, 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), and Mo (0.048) with EC 1.2 dS/m, pH 6.0. At 3 to 4 true leaf stage (40 DAS), the seedlings with Rockwool cubes were transplanted to the Rockwool slabs (1000 × 200 × 75 mm; Grodan Vital, Roermond, The Netherlands) at a density of 2.5 m^2 (0.5 m between and 0.5 m within-row). 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. Drip irrigation was automatically supplied with a total amount of 3.7–4.7 L per plant per day from 6:00 to 19:00 to maintain a minimum of 30–40%

efflux of the nutrient solution and avoid salt accumulation [23]. On a weekly basis, there was pruning of lower leaves and removal of side shoots once they were below the truss that was being picked. Flowers at anthesis were vibrated by an electronic toothbrush to stimulate pollination. Supplemental lighting was applied from 5:00 to 21:00 to compensate for low daily PARs.

2.2. Experimental Design and Root-Temperature Management

Two different root temperatures were considered in this study: a root-cooling treatment where the roots of tomato plants were kept at 10 °C and a control group (18–22 °C) consisting of non-treated plants at ambient temperature. The experiment contained 24 plants in total for two cultivars. There were six plants for each root-temperature treatment and each cultivar and two cultivars were randomly located in the greenhouse. Cooling mats (Clina Heiz und Kühlelemente GmbH, Berlin, Germany) with circulated cooled water from a thermostat (Julabo GmbH, Seelbach, Germany) were placed on the top and bottom of Rockwool slabs. Thermal isolation mats were covered to reduce heat transfer between Rockwool and ambient air. In the root-cooling group, the temperature of the thermostat was set at 10 °C and temperature loggers (developed by IBG2, Forschungszentrum Jülich, Jülich, Germany) were inserted in the middle of Rockwool slabs to record root temperature. Root temperature and the control group was not controlled. After the appearance of the first inflorescence (90 DAS), 10 °C root temperature was applied until the final harvest.

2.3. Harvest and Sample Preparation

Between 126 to 165 days after sowing (25 February to 5 April 2019), fruits from the first to the fourth cluster: I (base), II, III (middle), and IV (top) were successively harvested. Total yield per plant was the combination of the mass of all fruits from the first to fourth cluster per plant. Marketable yield was the combination of the mass of all healthy fruits above 20 g for 'Amoroso', while for 'Delioso', the minimum weight was 25 g. Fruits were harvested to measure diameter (equatorial and longitudinal diameter) and fresh weight and dry weight. The soluble solid contents of tomato fruit were determined using a refractometer (PAL-1, Atago, Tykyo, Japan). After the last harvest (165 DAS), plants were destructively harvested to measure stem height and diameter.

Six selected fruits from each cluster were collected for further biochemical analysis. Six fruits were quartered and the seeds and locular tissue were removed. Half of the fruits were pooled and quickly frozen in liquid nitrogen. The other half was dried at 65 °C until the weight stabilized. The frozen samples were ground in a kitchen coffee bean grinder (Clatronic International GmbH, Kempen, Germany) with liquid nitrogen, and then stored at −80 °C. The dried samples were ground in a mixer mill (MM400, Retsch GmbH, Haan, Germany).

2.4. Sugar Quantification

Glucose, fructose, and sucrose concentrations were determined by enzymatic analysis based on the method developed by Viola and Davies [24] with minor adjustments described by He et al. [22]. Briefly, homogenized frozen samples (50 mg) were subsequently suspended in 400 µL of ethanol (80%, v/v) and 400 µL of ethanol (50%, v/v). Each extraction was followed by an incubation for 15 min at 80 °C and a centrifugation at 13,200 rpm for 10 min. The clear supernatants were separated from the pellets and stored in ice. The pellets were then submitted to 200 µL of 80% ethanol (v/v) until the pellets were colorless. All supernatants were pooled together, directly measured or stored at −80 °C until further analysis. The concentrations of three sugars were measured at 340 nm using a spectrophotometer based on Viola and Davies [24] with slight modification [22]. Each sample was extracted and analyzed in duplicate and the results were reported as mg/g FW.

2.5. Carotenoids Determination

Lycopene and β-carotene were determined as previously described by He et al. [22]. Briefly, frozen powdered samples (25 mg) were suspended in 1200 µL pre-cooled acetone (VWR, Radnor, PA, USA), and centrifuged at 13,200 rpm for 15 min. The supernatant was filtered and homogenized with water and ethyl acetate. The mixture was then vortexed for 10 s and centrifuged for 15 min at 13,200 rpm. The new supernatant was transferred for HPLC analysis (1220, Agilent Technologies, Santa Clara, CA, USA). Separation of carotenoids was performed on a 250 × 4.6 mm RP C30 3.0 µm column (ProntoSIL, Bischoff Chromatography GmbH, Leonberg, Germany). Mobile phase consisted of 1 mM NH_4Ac (phase A) and methyl tert-butyl ether (phase B). The gradient solvent system was at a constant flow of 0.5 mL/min as follows: initial linear from 85% A to 70% A for 12 min, followed by 6 min at isocratic, followed by another linear from 70% to 15% for 5 min, and again 5 min at isocratic for 15% A, and then back to 85% A linearly in 5 min and stay isocratic for 5 min. Lycopene and β-carotene were detected at 475 and 450 nm by comparison with the standard curves made by authentic standards (DHI, Hørsholm, Denmark). Each sample was extracted and analyzed in duplicate. The concentrations were converted into µg/g FW and averaged.

2.6. Analysis of Organic Acid

Three organic acids: citric acid, malic acid, and ascorbic acid (vitamin C) were extracted and analyzed simultaneously by liquid chromatography–mass spectrometry (LC-MS) described by He et al. [22]. Briefly, frozen powdered samples (20 mg) along with internal standard $^{13}C_4$ malic acid (Sigma-Aldrich, St. Louis, MO, USA) were homogenized in 1200 µL extraction solvent containing 50 mg/L 1, 4 dithiothreitol and 50 mM ammonium acetate at 4 °C for 15 min and centrifuged for another 15 min. The supernatant was then filtered before injecting to Waters ACQUITY UHPLC system (binary pump, autosampler) coupled to a Waters Xevo TQ-S triple- quadrupole mass spectrometer (Waters Technologies Corp., Milford, MA, USA).

Separation of organic acids was achieved on a Nucleodur C18 Gravity-SB column (150 × 3 mm, 3 µM; Macherey-Nagel, Düren, Germany). The column was equipped with a column protection holder (Macherey-Nagel, Düren, Germany). The mobile phase was water (A) and acetonitrile (B) each containing 0.1% formic acid, at a flow rate of 0.6 mL/min. The gradient profile was run 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 MS conditions were set as described previously [22]. The concentration of each organic acid was qualified by comparison with the calibration of pure standard (Sigma-Aldrich, St. Louis, MO, USA). Each sample was extracted and analyzed in duplicate. Extract concentrations were converted into mg/g fresh weight and averaged.

2.7. Determination of Carbon, Nitrogen, Sulfur, and Other Elements

The dried and grounded sample was integrated by microwave after mixing with HNO_3, H_2O_2, and HF. After dilution, ICP-OES (Inductively Coupled Plasma with Optical Emission Spectroscopy, Agilent 7500ce, Waldbronn, Germany) was adopted to analyze P, K, Ca, Mg, and Fe. C and S concentrations of the sample were determined by infrared absorption (Leco CS 600, St. Joseph, MI, USA) based on the converted amount of CO_2 and SO_2 in flowing oxygen by radiofrequency heating. For the analysis of N, the samples were heated in flowing helium gas in a graphite crucible with resistance heating (Leco TCH 600, St. Joseph, MI, USA). Each sample was analyzed in duplicate and the concentration was expressed on the basis of dry weight.

2.8. Statistical Analysis

All statistical analyses were performed by the R studio version (1.2.1335). Each plant was regarded as one biological replicate and the cluster was regarded as repeated measures. Experimental results were expressed as the means ± standard deviation. On each cluster,

Student's *t* test was used. For cluster effect, one-way repeated measures ANOVA was used for the control and cool group.

3. Results

3.1. Climatic Parameters within Greenhouse

As shown in Figure 1, the average daily root temperature in the control group ranged from 18 to 22 °C, and it showed higher values between DAS 125 to 160. The maximum air temperature was stable, keeping at around 22 to 23 °C, and the values were above the root temperature in the control group. Average daily light intensity fluctuated during the entire growth period, with higher values around 60 µmol/m^{2}s after DAS 140.

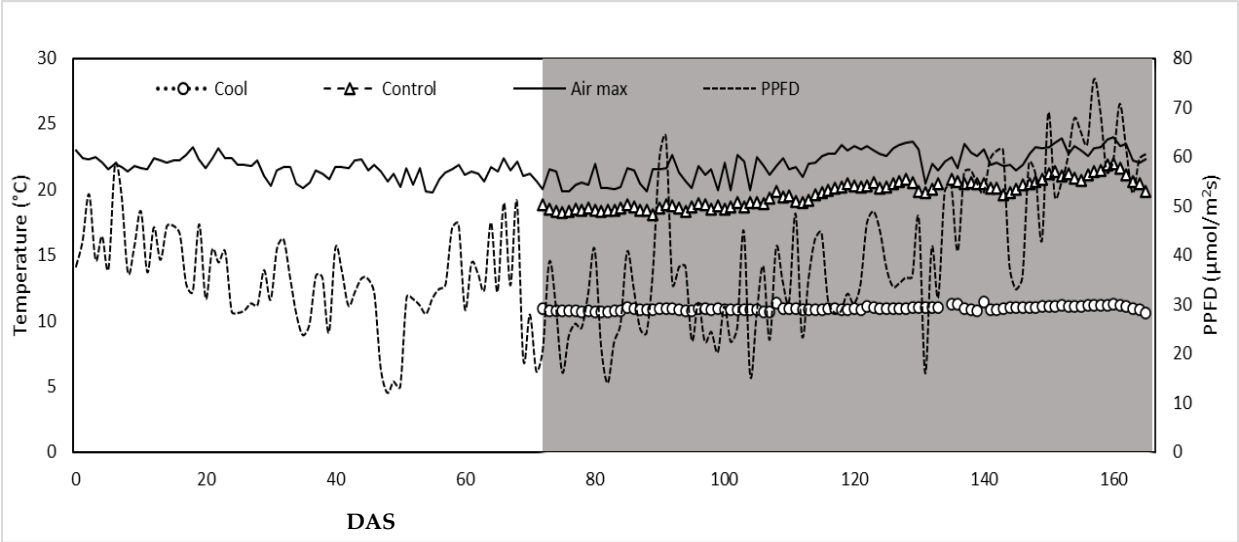

Figure 1. Moving average of daily maximum air temperature (Air_max), photosynthetic photon flux density (PPFD) recorded inside the greenhouse. Root-temperature treatments were indicated as grey. Root temperature from control group (open circles) and cooling group (open triangles) were recorded in the middle of Rockwool.

3.2. Plant Growth and Fruit Yield

Table 1 shows the plant growth parameters of two cocktail tomatoes at two root temperatures: yield, marketable yield per plant, diameter, and height of the shoot. However, none of these parameters were significantly affected by the root-temperature treatment, even though all four parameters showed a reduction in the cool group compared to the control group.

Table 1. Fruit yield and shoot growth parameters of cocktail tomatoes under the effects of two root-temperature treatments. Data are shown as the means ± standard deviation (n = 6).

	Treatment	Yield per Plant (g)	Marketable Yield per Plant (g)	Shoot Diameter (mm)	Shoot Height (cm)
'Amoroso'	Control	1580 ± 153	1570 ± 152	17.7 ± 0.7	206.5 ± 13.0
	10 °C	1557 ± 169	1551 ± 173	17.4 ± 0.5	198.7 ± 15.6
	S_T [b]	NS	NS	NS	NS
'Delioso'	Control	1705 ± 211	1672 ± 207	15.7 ± 0.4	213.1 ± 15.8
	10 °C	1699 ± 140	1669 ± 147	15.2 ± 0.5	220.6 ± 19.2
	S_T [b]	NS	NS	NS	NS

[b] Significance of differences between the control and root cooling (10 °C) samples (S_T) is given. NS, not significant. Student's *t* test was used.

Table 2 indicates the growth parameters in different clusters under the influence of root temperature. For 'Amoroso' plants, no significant changes in the fruit fresh weight

(FW), dry weight (DW), and diameters (equatorial and longitudinal) because of root cooling was observed in all clusters. Fruit water content (WC) and soluble solid content (SSC) responded to root-temperature treatment by showing a significant increase in the second cluster. On the other hand, the cluster position exhibited a change only in the equatorial diameter for both control and cool root groups.

Table 2. Average values of parameters related to the commercial quality of cocktail tomatoes of four cluster positions: I (base), II, III (middle), and IV (top) under the effects of two root-temperature treatments. Data are shown as the means ± standard deviation (n = 6).

	Cluster		FW (g)	DW (g)	WC (%)	Equ (mm)	Long (mm)	SSC (°Brix)
'Amoroso'	**I**	Control	354 ± 73.3	23.8 ± 9.01	93.4 ± 1.13	39.6 ± 1.85	33.6 ± 1.57	6.63 ± 0.58
		10 °C	345 ± 71.1	22.4 ± 6.66	93.6 ± 1.35	38.5 ± 2.09	32.8 ± 1.69	6.93 ± 0.32
		S_T [a]	NS	NS	NS	NS	NS	NS
	II	Control	403 ± 66.0	27.8 ± 6.20	93.1 ± 0.82	40.9 ± 1.13	34.3 ± 1.21	6.53 ± 0.24
		10 °C	415 ± 35.0	23.0 ± 2.27	94.5 ± 0.49	40.3 ± 0.88	34.2 ± 0.46	7.07 ± 0.50
		S_T [a]	NS	NS	*	NS	NS	*
	III	Control	412 ± 55.2	27.8 ± 3.23	93.4 ± 0.31	41.3 ± 1.60	34.3 ± 1.22	6.44 ± 0.21
		10 °C	410 ± 58.7	24.7 ± 5.38	93.8 ± 0.97	40.5 ± 2.10	34.0 ± 1.73	6.99 ± 0.76
		S_T [a]	NS	NS	NS	NS	NS	NS
	IV	Control	400 ± 25.4	26.2 ± 2.94	93.5 ± 0.47	41.9 ± 1.16	35.1 ± 0.83	6.31 ± 0.42
		10 °C	399 ± 78.9	25.1 ± 7.63	93.8 ± 1.00	41.1 ± 2.23	34.6 ± 1.84	6.96 ± 0.83
		S_T [a]	NS	NS	NS	NS	NS	NS
		SC_C [a]	NS	NS	NS	*	NS	NS
		$S10_C$ [a]	NS	NS	NS	*	NS	NS
'Delioso'	**I**	Control	353 ± 63.6	20.1 ± 3.72	94.3 ± 0.58	39.7 ± 1.2	32.3 ± 0.89	6.36 ± 0.32
		10 °C	350 ± 57.0	18.4 ± 1.93	94.6 ± 1.03	39.3 ± 1.51	32.2 ± 1.03	6.64 ± 0.29
		S_T [a]	NS	NS	NS	NS	NS	*
	II	Control	428 ± 50.8	26.4 ± 2.59	93.8 ± 0.38	41.5 ± 0.92	33.1 ± 0.92	6.69 ± 0.25
		10 °C	419 ± 42.2	25.1 ± 2.68	94.0 ± 0.38	41.9 ± 1.1	33.8 ± 1.09	6.83 ± 0.18
		S_T [a]	NS	NS	NS	NS	NS	NS
	III	Control	434 ± 63.3	27.3 ± 4.39	93.7 ± 0.12	42.9 ± 1.59	34.0 ± 1.12	6.66 ± 0.32
		10 °C	417 ± 41.7	25.6 ± 3.62	93.9 ± 0.57	41.9 ± 1.45	33.6 ± 1.41	6.79 ± 0.46
		S_T [a]	NS	NS	NS	NS	NS	NS
	IV	Control	489 ± 64.1	28.5 ± 4.10	94.1 ± 0.69	43.3 ± 1.23	34.7 ± 1.05	6.40 ± 0.42
		10 °C	513 ± 56.6	30.9 ± 4.38	94.0 ± 0.58	43.3 ± 1.65	34.5 ± 1.18	6.64 ± 0.47
		S_T [a]	NS	NS	NS	NS	NS	NS
		SC_C [a]	***	***	NS	***	***	NS
		$S10_C$ [a]	***	***	NS	***	***	NS

[a] Significance of differences between the control and root cooling (10 °C) samples (S_T), cluster position in the control samples (SC_C), and cluster position in the root cooling (10 °C) samples ($S10_C$) is given. FW: fresh weight of cocktail tomatoes from the corresponding cluster position, DW: dry weight, WC: water content, Equ: equatorial diameter, Long: longitudinal diameter, and SSC: soluble solid content. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS, not significant. On each cluster, Student's *t* test was used. For cluster effect, one-way repeated measures ANOVA was used for the control and cool group.

For 'Delioso' plants, root temperature only exhibited a significant effect on fruit SSC. Lower root temperature induced an increase in sugar levels, and this has been significantly shown in the values of the first cluster. Considering the cluster effect, FW, DW, and both diameters (Equ and Long) of the cocktail tomato fruits were closely related to both control and cooling root groups. The fourth cluster accumulated the higher biomass in FW and DW and bigger fruits in size.

3.3. Bioactive Compounds

The results of bioactive compounds including three soluble sugars, two carotenoids, and three organic acids under the influence of root temperature and cluster positions are shown in Figures 2–4. For 'Amoroso' plants, in the second cluster, fruits of the root-cooling group accumulated more glucose, fructose, and ascorbic acid, as shown in Figures 2a,b and 4c. In the first and third cluster, fruits of the root-cooling group contained

20.0% and 34.6% higher lycopene levels compared to the control group. Around 13.5–15.4% lower malic acid concentration can be observed in the fruits of all the four clusters of the root-cooling group (10 °C), but not significantly. With regards to the effect of cluster position, fructose, lycopene, and β-carotene concentration of fruits showed significant differences in the control group, which were not observed in the cool group. However, the levels of glucose, sucrose, citric acid, and ascorbic acid were significantly related to the cluster positions in both groups. In the case of sucrose, the first cluster has the highest value in both control and cooling group. In addition, the levels of citric acid and ascorbic acid decreased from the first cluster to the fourth cluster.

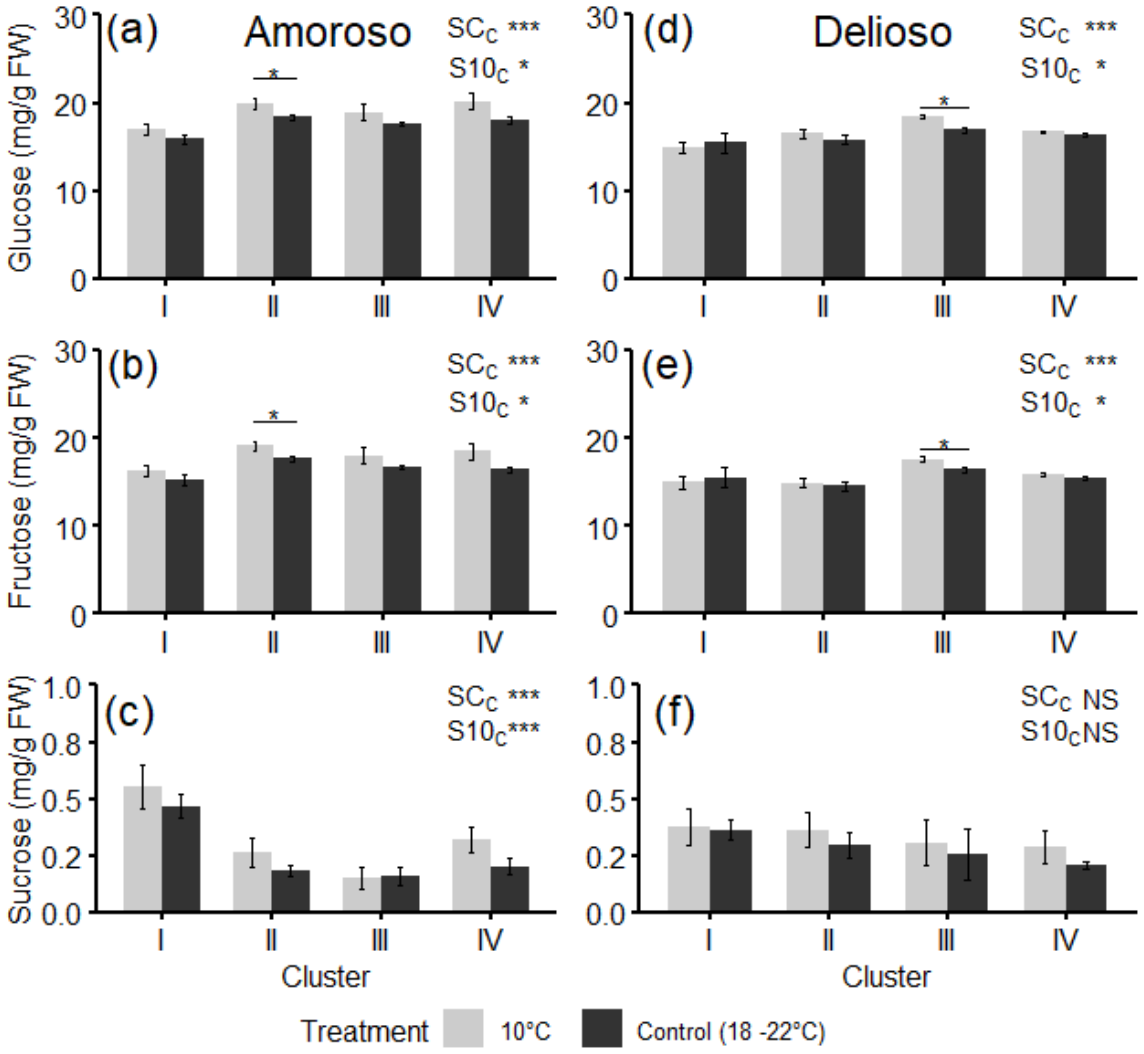

Figure 2. Average values of glucose (**a**,**d**), fructose (**b**,**e**) and sucrose (**c**,**f**) concentration of cocktail tomatoes of four cluster positions: I (base), II, III (middle), and IV (top) under the effects of two root-temperature treatments. Vertical bars indicate standard deviations of six replicates. Significance of differences between the control and root cooling (10 °C) samples (S_T), cluster position in the control samples (SC_C), and cluster position in the root cooling (10 °C) samples ($S10_C$) is given. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS, not significant. On each cluster, Student's t test was used. For cluster effect, one-way repeated measures ANOVA was used for the control and cool group.

In 'Delioso' fruits, as shown in Figures 2d,e and 3c, root cooling exhibited a positive effect on the glucose, fructose, and ascorbic acid of the third cluster, and lycopene of the second and fourth clusters. In the third cluster, glucose and fructose increased by 8.7% and 7.2% in the root-cooling group, respectively, compared to the control group. For the second and fourth truss, root cooling increased lycopene concentration by 19.4% and

10.4%, respectively, compared to no treatment. As for the cluster position effect, statistical differences were observed in the levels of glucose and fructose of the cooling group, rather than the control group. The third cluster of the cooling group contained the highest sugar concentration compared to other trusses. There were significant differences in carotenoids and organic acids in both control and cool groups. Specifically, the highest lycopene and β-carotene level was observed in the fourth cluster and the highest acid concentration was in the second cluster.

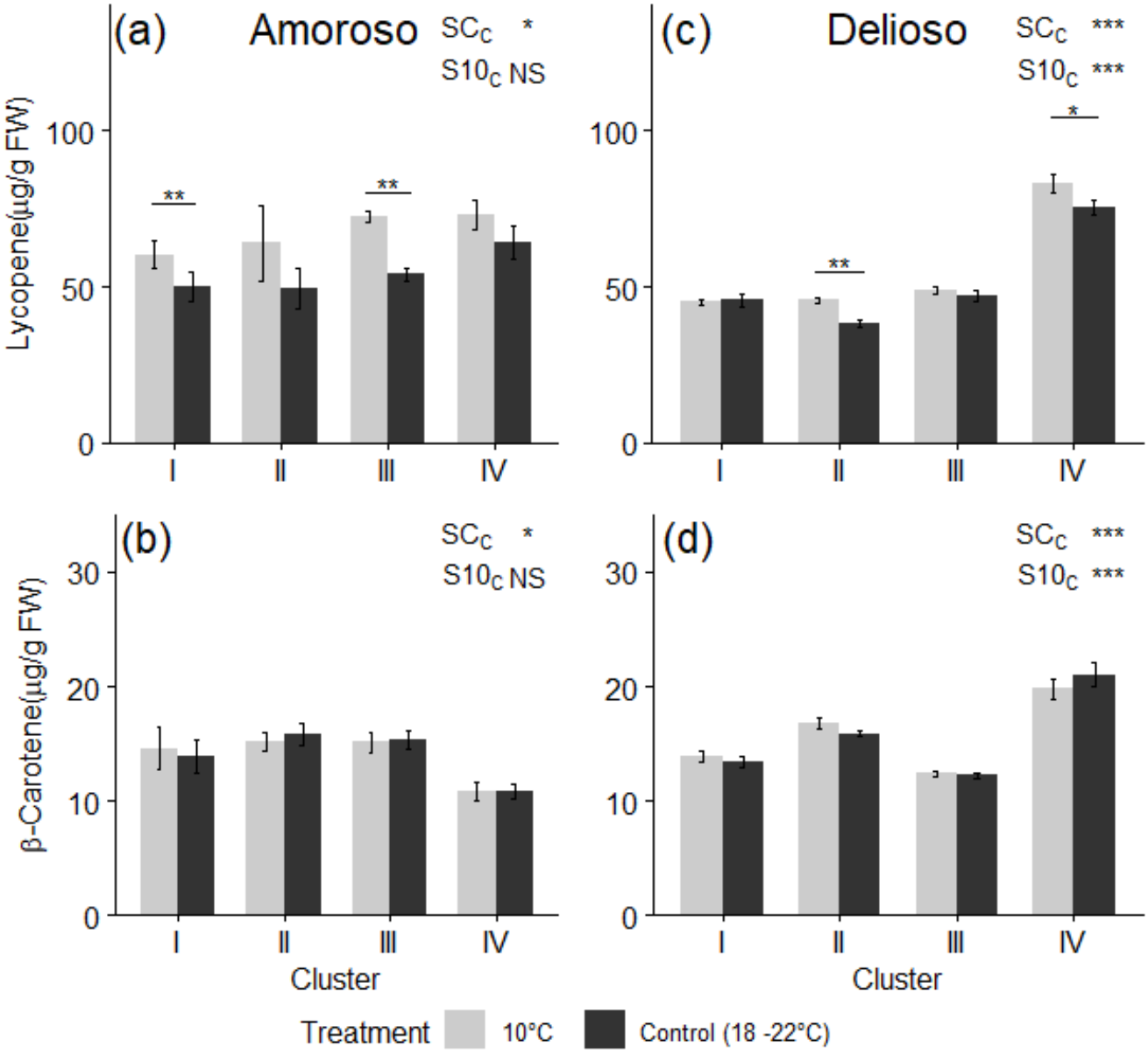

Figure 3. Average values of lycopene (**a**,**c**) and β-carotene (**b**,**d**) concentration of cocktail tomatoes of four cluster positions: I (base), II, III (middle), and IV (top) under the effects of two root-temperature treatments. Vertical bars indicate standard deviations of six replicates. Significance of differences between the control and root cooling (10 °C) samples (S_T), cluster position in the control samples (SC_C), and cluster position in the root cooling (10 °C) samples ($S10_C$) is given. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS, not significant. On each cluster, Student's *t* test was used. For cluster effect, one-way repeated measures ANOVA was used for the control and cool group.

3.4. Minerals

As shown in Table 3, a significant positive effect of root cooling was found in C concentration of 'Amoroso' fruits. N and Ca concentrations reacted statistically to the cluster position in the control group, whereas the significant effect of Ca levels was not observed in the control group. Generally, N concentration was reduced from the first cluster to the fourth cluster gradually. Ca concentration, in contrary, was the highest in the fruits of the fourth cluster.

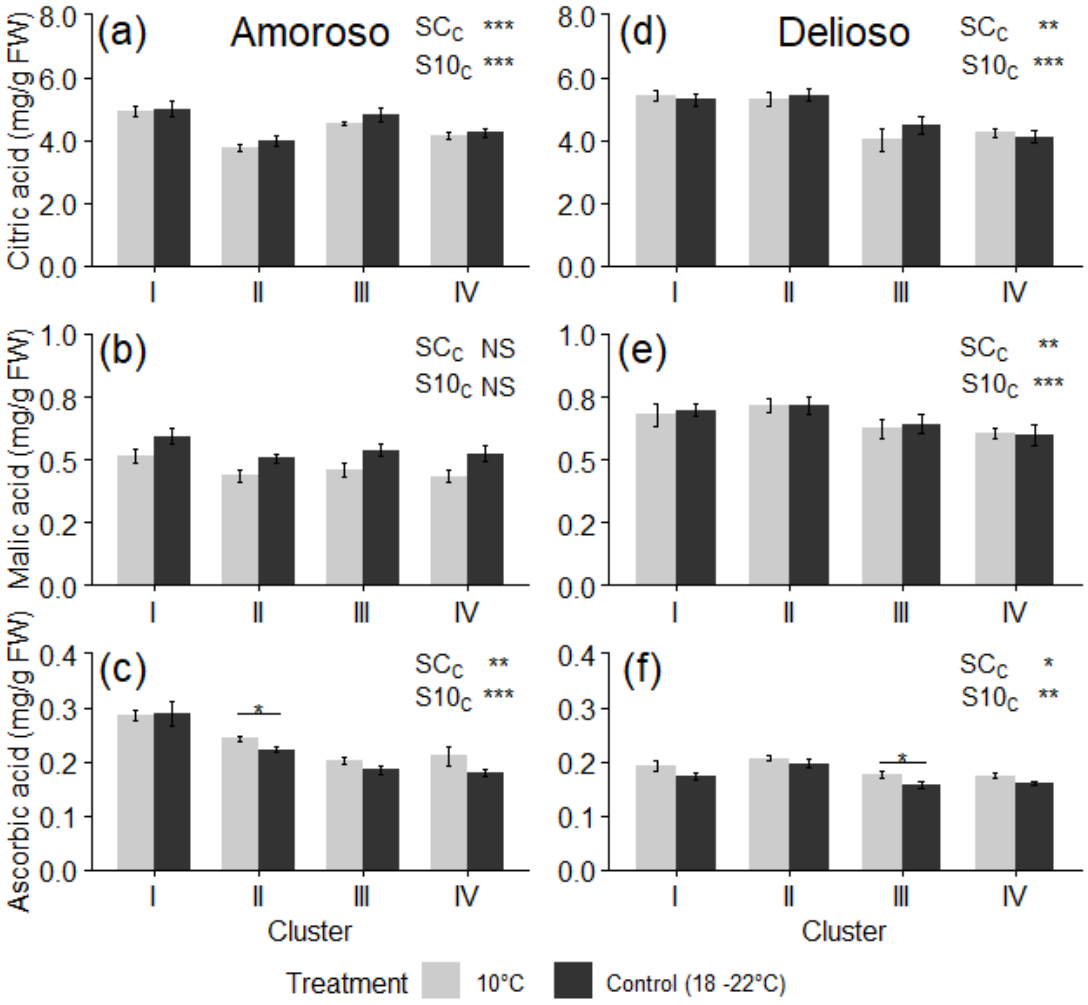

Figure 4. Average values of citric acid (**a**,**d**), malic acid (**b**,**e**) and ascorbic acid (**c**,**f**) concentrations of cocktail tomatoes of four cluster positions: I (base), II, III (middle), and IV (top) under the effect of two root-temperature treatments. Vertical bars indicate standard deviations of six replicates. Significance of differences between the control and root cooling (10 °C) samples (S_T), cluster position in the control samples (SC_C), and cluster position in the root cooling (10 °C) samples ($S10_C$) is given. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS, not significant. On each cluster, Student's t test was used. For cluster effect, one-way repeated measures ANOVA was used for the control and cool group.

For 'Delioso' fruits, only K concentration in the fruits of the second cluster and Zn in the first cluster was decreased by 10.0% and 17.6%, respectively, because of root cooling compared to the control group. In the case of cluster effect, N, P, Mg, and K concentrations of fruits were markedly negatively correlated with the cluster position in both control and cool groups.

Table 3. Average values of the mineral concentration of cocktail tomatoes of four cluster positions: I (base), II, III (middle), and IV (top) under the effects of two root-temperature treatments. Data are shown as the means ± standard deviation (n = 6).

	Cluster		C (%)	N (%)	P (%)	Na (mg/kg)	Mg (%)	K (%)	Ca (%)	Zn (mg/kg)
'Amoroso'	I	Control	42.5 ± 0.58	2.05 ± 0.21	0.42 ± 0.05	127 ± 30.5	0.09 ± 0.02	3.49 ± 0.47	0.04 ± 0.01	17.5 ± 2.57
		10 °C	42.5 ± 0.55	2.05 ± 0.10	0.44 ± 0.04	135 ± 28.8	0.09 ± 0.01	3.60 ± 0.29	0.04 ± 0.01	17.7 ± 2.17
		S_T [a]	NS	NS	NS	NS	NS	NS	NS	NS
	II	Control	**42.3 ± 0.48**	1.88 ± 0.15	0.38 ± 0.04	107 ± 16.4	0.07 ± 0.01	3.21 ± 0.27	0.03 ± 0.01	15.8 ± 2.21
		10 °C	**43.1 ± 0.29**	1.99 ± 0.21	0.40 ± 0.06	114 ± 19.2	0.08 ± 0.01	3.33 ± 0.39	0.03 ± 0.01	15.3 ± 2.16
		S_T [a]	*	NS	NS	NS	NS	NS	NS	NS
	III	Control	42.9 ± 0.32	1.93 ± 0.09	0.40 ± 0.03	123 ± 19.4	0.08 ± 0.01	3.56 ± 0.24	0.04 ± 0.01	18.4 ± 2.83
		10 °C	42.6 ± 0.38	1.79 ± 0.30	0.38 ± 0.04	120 ± 10.6	0.08 ± 0.01	3.40 ± 0.37	0.04 ± 0.01	16.4 ± 3.82
		S_T [a]	NS	NS	NS	NS	NS	NS	NS	NS
	IV	Control	42.3 ± 0.97	1.70 ± 0.27	0.36 ± 0.05	117 ± 17.7	0.08 ± 0.02	3.13 ± 0.43	0.05 ± 0.01	17.2 ± 2.78
		10 °C	42.1 ± 0.97	1.68 ± 0.18	0.37 ± 0.05	109 ± 15.9	0.07 ± 0.01	3.02 ± 0.42	0.04 ± 0.01	16.1 ± 2.26
		S_T [a]	NS	NS	NS	NS	NS	NS	NS	NS
		SC_C [a]	*	***	NS	NS	NS	NS	*	NS
		$S10_C$ [a]	NS	*	NS	NS	NS	NS	NS	NS
'Delioso'	I	Control	41.9 ± 0.51	1.82 ± 0.08	0.43 ± 0.02	152 ± 27.6	0.10 ± 0.01	4.06 ± 0.13	0.04 ± 0.01	**17.1 ± 1.65**
		10 °C	41.9 ± 0.53	1.91 ± 0.25	0.43 ± 0.03	150 ± 19.9	0.10 ± 0.01	4.05 ± 0.30	0.04 ± 0.01	**14.0 ± 2.03**
		S_T [a]	NS	NS	NS	NS	NS	NS	NS	**
	II	Control	42.2 ± 0.60	1.74 ± 0.11	0.35 ± 0.03	135 ± 22.9	0.08 ± 0.01	**3.68 ± 0.24**	0.03 ± 0.01	14.9 ± 2.23
		10 °C	42.5 ± 0.54	1.80 ± 0.14	0.38 ± 0.03	134 ± 20.4	0.09 ± 0.01	**3.31 ± 0.15**	0.03 ± 0.01	14.0 ± 1.55
		S_T [a]	NS	NS	NS	NS	NS	*	NS	NS
	III	Control	42.2 ± 0.69	1.66 ± 0.07	0.35 ± 0.02	134 ± 10.2	0.09 ± 0.01	3.47 ± 0.16	0.04 ± 0.01	16.3 ± 1.43
		10 °C	42.1 ± 0.52	1.62 ± 0.15	0.35 ± 0.02	143 ± 13.0	0.09 ± 0.00	3.48 ± 0.10	0.04 ± 0.01	15.7 ± 1.68
		S_T [a]	NS	NS	NS	NS	NS	NS	NS	NS
	IV	Control	42.3 ± 0.38	1.62 ± 0.12	0.34 ± 0.02	123 ± 12.9	0.08 ± 0.00	3.47 ± 0.24	0.04 ± 0.01	15.0 ± 2.35
		10 °C	42.4 ± 0.21	1.57 ± 0.08	0.34 ± 0.01	133 ± 8.0	0.08 ± 0.00	3.15 ± 0.25	0.04 ± 0.01	15.6 ± 2.71
		S_T [a]	NS	NS	NS	NS	NS	NS	NS	NS
		SC_C [a]	NS	**	***	NS	*	***	NS	NS
		$S10_C$ [a]	NS	***	***	NS	**	***	NS	NS

[a] Significance of differences between the control and root cooling (10 °C) samples (S_T), cluster position in the control samples (SC_C), and cluster position in the root cooling (10 °C) samples ($S10_C$) is given. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS, not significant. On each cluster, Student's *t* test was used. For cluster effect, one-way repeated measures ANOVA was used for the control and cool group.

4. Discussion

Two cocktail tomato cultivars grown in hydroponic culture were exposed to two different root temperatures: 10 °C and control (18 to 22 °C), after the first inflorescence. Several studies have reported that suboptimal root temperature reduced the growth rate and the biomass. For example, a study reported that at lower root temperature, all plant growth of *Agastache rugosa* was decreased [11]. Our previous study also reported the negative effect of root cooling on cocktail tomato yield in winter [22]. In many species, root temperature below the optimum range is often related to a reduction in shoot growth yield [25]. This could be caused by a number of factors, such as limited water and nutrient uptake due to drought stress [26], reduced photosynthesis rate, alteration of plant hormone signaling [27], and reallocation of carbon [28,29]. However, in our current studies, total yield and total marketable yield per plant were not affected by root-temperature treatment. Additionally, water content of 'Amoroso' fruits even increased at lower root temperature, especially for the second cluster. Therefore, drought stress due to limited water uptake at lower root temperature in our studies may not be the case. In the experiment of Fujimura et al. [30], the tomato plants showed acclimation to root cooling at 12 °C within one week by showing unaffected photosynthesis rate and stomatal conductance. Therefore, the unchanged yield and increased water content in the second cluster of 'Amoroso' at lower root temperatures of our study could be caused by long-term cold acclimation since we applied root-cooling treatment from the first florescence to the final harvest.

Fruit position within the plant appears to be an important factor in determining fruit yield and composition. In crops such as apple, kiwifruit, and cucumber, the shape of fruits was predetermined by the position within the plant [31]. Pannico et al. [32] observed that the kernel fresh and dry weight of hazelnuts increased linearly with fruit height above the ground. Similarly, the fruit size of peaches decreased from the top to the bottom of the trees and light distribution within the canopy could be the determinant influencing factor [33,34]. In agreement with previous study, the FW and DW of 'Delioso' fruits increased with the

number of the clusters. Even for 'Amoroso', FW of the second and third cluster were higher than the first cluster. In addition, the cocktail tomato fruits from the upper truss were bigger with longer equatorial and longitudinal diameter. However, in another study with nine genotypes of tomato, fruit weight and diameter were highest in the first cluster and reduced linearly to the fourth cluster [35]. Most of the research focused on the fruit position within the cluster and reported that fruit weight reduces from proximal to distal fruits within the cluster due to carbon competition among the fruit sink [19,36]. Instead of the fruit competition, the main differences in fruit weight within the tree of our studies could be the light intensity. Since the light source in our experiment was above the tomato plants, the results could be related to C-source limitation due to the low light intensity reaching leaves very close to the fruit, the low photosynthetic capacity of these leaves, and an insufficient area of these leaves [32,33].

The characteristic sweet–sour taste and flavor of tomato are mainly due to the reducing sugars (mainly glucose and fructose), free acids (mainly citric acid and malic acid), and their ratio [37]. In addition to their importance in taste, soluble sugars play an important role in regulating carbohydrates when plants are exposed to abiotic stress [38]. Sugar accumulation at low root temperature has been widely reported in various species, such as lettuce [39], red leaf lettuce [40], spinach [41], and Chinese broccoli [9,42]. Our previous studies on cocktail tomatoes have also shown that glucose and fructose concentration increased by root cooling regardless of winter or summer cultivation [22]. In the present study, an increase in glucose and fructose concentration in the second cluster of 'Amoroso' and the third cluster of 'Delioso' agreed with previous findings. Increased sugar levels can be regarded as a defensive mechanism of the plant at cold stress since sugars can act as osmoprotectants, nutrients, primary stress messengers and scavengers [43]. Cucumber seedlings had been found to contain higher sugar concentrations at 12 °C than 20 °C root temperature [44]. Also, at lower root temperature, root respiration and energy consumption are reduced, and, subsequently, more carbohydrates are redistributed to the shoot, including fruits [27].

Citric acid and malic acid are the two major organic acids in tomato fruits [45], which are responsible for the total acidity [46]. In Shaw's previous reports, the total acidity was more controlled by genetic traits instead of climatic factors [47]. Fujimura et al. [30] has also reported that the levels of citric acid and malic in tomato fruit acid did not respond to root cooling. In agreement with the previous studies, malic acid and citric acid concentrations of 'Amoroso' and 'Delioso' fruits were not affected by root cooling in this study. However, vitamin C (ascorbic acid) concentration was increased in the second cluster of 'Amoroso' fruits and the third cluster of 'Delioso' fruits. This result of vitamin C agreed with Hidaka et al. [48] and He et al. [22], who proved that the increment of ascorbic acid concentration is the protection mechanism under low temperature induced oxidative stress.

Lycopene and β-carotene are the predominant carotenoids existing in the tomato fruits, which are responsible for the red and orange color, respectively [49]. Together with ascorbic acid, lycopene and β-carotene are important antioxidants in tomato fruits. The concentrations of these two carotenoids are dependent on many factors, including cultivars, ripeness, environmental factors, agricultural practices, and post-harvest conditions [36,50–54]. In our studies, more lycopene was accumulated in the fruits of both cultivars when the roots were immersed in the lower temperature, which is consistent with our previous research about root temperature stress [22]. Lower root temperature causes stress for plants, which leads to the accumulation of reactive oxygen species (ROS), such as $^1O_2$, O_2^-, H_2O_2 and •OH [55]. To counteract the deleterious oxidative damage of ROS, plants have to produce antioxidant enzymes and antioxidants, such as ascorbates and carotenoids, as their defense systems [56]. Eventually, various metabolites accumulate in the plants under root-temperature stress. For example, in red romaine lettuce and red leaf lettuce, 10 °C root temperature accelerated the accumulation of anthocyanin and phenols than other temperatures [40,57]. In two medicinal plants, *Catharanthus roseus* and *Nicotianna tabacum*, the biosynthesis and accumulation of alkaloids were increased by altering root temperature during 48 h root-temperature treatment [58]. In agreement with previous studies, higher

concentrations of ascorbic acid, lycopene, and β-carotene were observed as a function of root cooling in our experiments.

Bioactive compounds of fruits differed among the different positions within the plants. In other plants, sugar and ascorbic acid levels of peach fruits decrease from the top to the bottom of the tree [59]. Similar patterns have been described for unsaturation of fatty acids in the kernel of hazelnut trees [32]. This variability has often been explained by the inhomogeneous light intensity and distribution within the tree [60]. In tomato plants, Fanasca et al. [61] found that lycopene content was lower and β-carotene content was higher in the tenth truss compared to the fifth truss and attributed this difference to the higher temperature during ripening of the tenth truss. Similarly, ascorbic acid content of tomato fruits increased progressively from the first to the following truss, which was highly influenced by the light intensity [62]. By contrast, Akhtar et al. [35] found that soluble solids content, β-carotene and lycopene did not statistically vary in the first five clusters of nine tomato genotypes fruits. In this study, more glucose, fructose, lycopene, and β-carotene were accumulated in the higher clusters of 'Delioso' in the control group, whereas the differences were insignificant in the root-cooling group. Regarding 'Amoroso' fruits, higher glucose and fructose concentrations were also observed in the higher cluster, but only in the root-cooling group. Root temperature treatment changed the pattern of bioactive compounds in different cluster positions and these alterations depended on different genotypes. Therefore, besides light intensity, the levels of sugars, carotenoids, organic acids, and other secondary metabolites in the fruits are dependent on many other factors, such as agronomic, seasonal, climatic, or genotype [63,64].

Carbon is the skeleton of the fruits and its concentration is the result of carbon fixation, partitioning, and respiration [65]. Pollock and Eagles [66] reported that the low temperature on carbon fixation and translocation is relatively mild. In the current studies, we found that the carbon concentration increased at lower root temperature only in the second cluster of 'Amoroso' fruits. It is believed this carbon accumulation in shoots was due to decreased sink demand by the cold root or cold grinding effect on the phloem pathway [67,68]. Therefore, the impact of root temperature on carbon levels could be the result of combined effects of fixation, translocation, and respiration.

The level of macro- and micro-elements in the tomato fruits are important dietary sources of essential elements. The cationic minerals in the fruits are influenced by root uptake, transport from root to shoot and dilution effect caused by growth [69]. It is generally believed that the uptake of nutrients was reduced at lower root temperature, especially in the short term, for example, grapevine [70], rice [71], and spring barley [72]. This could be due to altered root morphology [67,73,74], limited energy available caused by reduced root respiration [40], and hampered root hydraulic conductivity [75]. In addition to root uptake, translocation from root to shoot through xylem is affected by root temperature. Petterson [76] found that K, N, and Mg tended to accumulate in the roots of barley at 10 °C. Aidoo et al. [77] subjected pepper plants to a low root-zone temperature and found that more N was allocated to roots to ensure the survival of the whole plant. In our present studies, only Zn and K concentrations in the fruits of 'Delioso' indicated a significant reduction at lower root temperature. This could be due to reduced translocation rate and uptake rate. Most cationic elements of two cocktail tomato cultivars did not make changes after root cooling. Janska et al. [78] reported that plants recover and adapt to the new thermal conditions after the extension of cooling time. Considering the extended root cooling in our experiment, the unchanged element concentrations in the fruits could be the result of cold acclimation.

As another major fruit flesh composition, mineral content has also been reported to vary largely within the tree. For example, in kiwifruits, fruits at positions near the trunk of vines had higher Ca concentrations than fruits at more distal positions [79]. Mandarin fruit flavedo accumulated significantly higher concentrations of Ca and Mg outside the canopy and significantly higher levels of K inside the canopy [80]. This variability of minerals could be attributed to the microclimate within the plants, such as light or humidity [80]. In

our study, N concentration of both cultivars, and P, Mg, K concentration of 'Delioso' were reduced from the first cluster to the fourth cluster. Ca levels of 'Amoroso' increased from the first to the fourth cluster. These results suggested that xylem was the main vasculature supply conduit to the tomato fruit for mineral nutrients [80]. In addition, carbon and calcium of 'Amoroso' fruits showed significant differences to different cluster positions only in the control group, but not in the cool group. Again, root temperature altered the behavior of carbon and calcium among the four clusters.

5. Conclusions

In conclusion, cooling roots at 10 °C during winter had a beneficial effect on the accumulation of bioactive compounds such as glucose, fructose, lycopene, and ascorbic acid without reducing fruit yield and growth for both cultivars. Furthermore, most minerals of both cultivars remained unaffected values to root cooling and only K and Zn levels of 'Delioso' were reduced in the cooling group compared to control group. Specifically, the fruits from the second or third clusters were more influenced by root cooling with more sugars, lycopene, and ascorbic acid. In addition, the effect of cluster positions on fruit quality was different in two root temperature groups. The heterogeneity in fruit quality was reduced by root cooling in 'Amoroso' and, however, was increased in 'Delioso' compared to the changes in the control group. Considering the low temperature of underground water in winter, irrigation with underground water to the roots of tomato plants would be an alternative to ensure high-quality tomatoes and sustainable greenhouse production.

Author Contributions: Conceptualization, F.H. and A.J.K.; Formal analysis, F.H.; Funding acquisition, U.S.; Investigation, F.H.; Methodology, F.H., B.T. and A.J.K.; Project administration, B.T.and A.J.K.; Supervision, B.T., T.K. and A.J.K.; Visualization, F.H.; Writing—original draft, F.H.; Writing—review and editing, F.H., B.T., A.J.K. and T.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was partly funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy—EXC 2070—390732324 (PhenoRob—Robotics and Phenotyping for Sustainable Crop, www.phenorob.de).

Acknowledgments: We wish to acknowledge helpful discussion with Andreas Ulbrich. Many thanks to Beate Uhlig, Katharina Wolter-Heinen, Thorsten Brehm, Esther Breuer, and Andrea Neuwohner, for their generous support and excellent advice on technical in the greenhouse and lab work. We thank ZEA-3 of Forschungszentrum Jülich for their technical support in mineral analysis.

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

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