

## Research Article

# Effects of Root Cooling on Plant Growth and Fruit Quality of Cocktail Tomato during Two Consecutive Seasons

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Understanding the effects of root temperature on plant growth and key food components of horticultural crops under greenhouse conditions is important. Here, we assess 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. Plants were grown hydroponically on rockwool under different root temperatures (16–27°C and 10°C) from the 2<sup>nd</sup> inflorescence to harvest inside the greenhouse. A root temperature of 10°C was controlled independently from air temperature (18–23°C in winter and 21–29°C in summer) by circulating cooling water. Reductions of marketable yield per plant (7.9–20.9%) in both cultivars were observed in response to root cooling in winter, but not significantly in summer. In most cases, root cooling had a positive effect on the functional quality (sugars, vitamin C, and carotenoids levels). In the case of “Delioso,” glucose concentration increased by 7.7–10.3%, vitamin C by 20–21%, and lycopene by 16.9–20.5% in both seasons. “Amoroso” exhibited only higher consistent values in glucose with increments between 6.9 and 7.8% in the two seasons. The levels of elements decreased by root cooling, with statistically significant reduction of N, P, S, and Fe by 12.1–15.7% in “Delioso” in winter and P and Zn by 9.1–22.2% in both cultivars in summer. Thus, manipulation of root temperature could be a feasible method to improve the overall fruit quality of cocktail tomato; however, this effect was also dependent on cultivars and other environmental factors.

## 1. Introduction

Tomato (*Lycopersicon esculentum* Mill.) is an important horticultural crop worldwide with 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 [1]. Health promoting effects as well as potential risk of tomatoes and tomato-based product consumption for humans are well known and have been reviewed by Salehi et al. [2]. Hence, the protective action is typically assigned to significant levels of antioxidants such as vitamin C [3], lycopene [4], or carotenoids [5]. Cocktail tomatoes with an average weight of 20–50 g are perceived as tastier by consumers [6], and

because of 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 [7] because of its higher skin-to-volume ratio [8].

Among the environmental factors, temperature plays a crucial role in the growth of tomato plants and development of fruits. At suboptimal air temperature for the vegetative stage, tomato seedlings tend to produce larger cells to store more starch, indicating thicker leaves and relative lower growth rate [9]. Even short periods of low temperatures could induce blossom-end scarring of fruits, making them sensitive to bruising and serve as possible entrance for postharvest diseases [10]. During flower development stage,

cooler air temperature induced an increase in the number of flowers, late ripeness, and eventually larger fruits [11, 12]. The optimal air temperature for fruit setting is 18–20°C, and a temperature higher than 30°C causes fruit cracking and blotchy ripening [13]. During fruit development stages, accumulation of carotenoids is promoted above 10°C, but inhibited above 30°C air temperature [14, 15].

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 [16, 17]. 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 [18–23]. Cooper [24] 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 [25, 26], 25–30°C during flower differentiation, and 15–30°C during fruit development [26]. Root temperature also influences the distribution of carbohydrates between shoot and root by modifying the sink strength of root [27–29]. The shoot-to-root ratio of tomato is positively correlated with root temperature [30].

The overall flavor of tomato is largely determined by the concentration of sugars and acids [31]. 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 [32] and spinach [33]. Carotenoids of tomato are an important source for human nutrition due to high frequency in the diet [34]. A number of environmental factors, such as light intensity, CO<sub>2</sub> levels, salinity, and temperature, are known to influence the levels of carotenoids in tomato [15, 34]. Lower root temperature was also demonstrated recently to enhance the accumulation of carotenoids, such as  $\beta$ -carotene, in hydroponically grown carrots [35]. 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 the leaves [33]. Tomato fruits also supply essential elements for human health, such as K, Ca, P, and Mg [36]. 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 [37]; Ca, Mg, P, K, Fe, and Mn of African snake tomato [38]; Fe of spinach [33]; K, N, P, Ca, and Mg of young tomato plants [22]. Thus, proper manipulation of root temperature could improve the taste and health components, leading to increased crop market value.

Maintaining the root temperature in the optimal range has been used an energy-efficient method to alleviate injury caused by suboptimal air temperature [39]. Trudel and Gosselin [40] and Gosselin and Trudel [41] reported that root temperatures lower than 16°C greatly reduced the yield

of tomato, while warming the roots partially alleviate cool air temperature in the night by showing a rise in yield. Kawasaki et al. [22] 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) [42]. Furthermore, roots growth and nutrient uptake of young tomato plants were enhanced by root cooling at higher air temperatures by production of auxin [21]. 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 water stress at the late stages of plant development improved the overall fruit quality without reducing yield [43, 44]. In line with the findings under water stress, application of root cooling after the 2<sup>nd</sup> anthesis only may, however, improve the fruit quality without decreasing the yield. We hypothesized that such conditions reduce root sink strength for photo-assimilates and therefore favor the translocation of carbohydrates to the growing fruits. 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 [45]. To test this hypothesis in cocktail tomato, a soilless culture (rockwool) was carried out in the two seasons (2017–2018 winter and 2018 summer). At the start of the 2<sup>nd</sup> 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  $\beta$ -carotene), and elements (macro and micro) were measured after harvest.

## 2. Materials and Methods

**2.1. Plant Material and Growth Conditions.** Cocktail tomato cv “Delioso” and cv “Amoroso” were provided from Rijk Zwaan breeding company (The Netherlands). Seeds were sown on 11<sup>th</sup> October 2017 and 10<sup>th</sup> April 2018 into rockwool plugs (25 × 25 × 40 mm 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 the temperature was kept at 25°C. At 3–4 DAS (days after sowing), the seeds were germinated, and the lid was removed. After the 1<sup>st</sup> true leaf was developed (around 15 DAS), 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 and 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 × 75 mm; 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 and EC 2.8 dS/m. 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 [46]. 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<sup>2</sup> (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 7<sup>th</sup> cluster. Flowers at anthesis were vibrated by electronic toothbrush to stimulate pollination. Six supplementary high pressure discharge lamps (720  $\mu$ mol/s) (MGR-K400, DH LICHT, Germany) were open 16 h from 6.00 to 22.00 in the two seasons to compensate 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. 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 Jülich) 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 2<sup>nd</sup> inflorescences on 3<sup>rd</sup> January (84 DAS) and 6<sup>th</sup> June 2018 (62 DAS), 10°C root temperature was applied until the final harvest.

**2.3. Harvest and Sample Preparation.** Harvest of fruits was done between 28<sup>th</sup> February and 6<sup>th</sup> April and 20<sup>th</sup> July and 29<sup>th</sup> August 2018. After starting the treatment after the 2<sup>nd</sup> inflorescence, only the fruits from the 2<sup>nd</sup> to 5<sup>th</sup> 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 2<sup>nd</sup> to 5<sup>th</sup> cluster per plant. Marketable yield was the combination of the mass of all healthy, red and ripe fruits above 20 g for “Amoroso,” while for “Delioso,” the minimum weight was 25 g. Mean fruit weight was approximated by dividing total yield by total number of fruits per plant. After the fruits of the 7<sup>th</sup> cluster were harvested, plant shoot length was also measured. Diameter at the base and internode between every two clusters was measured and averaged. After harvest, equatorial and

longitudinal diameter (mm) and fresh weight (g) of the fruits were measured.

Two randomly selected red and ripe fruits from each part of two clusters (the 2<sup>nd</sup> and 3<sup>rd</sup>) were used 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 mixer mill (MM400, Retsch, Germany).

**2.4. Sugar Quantification.** Sugar concentrations were determined by enzymatic analysis according to Viola and Davies [47], with minor adjustments. To 50 mg of frozen samples 400  $\mu$ l 80% (v/v) ethanol was added, incubated at 80°C for 15 min and centrifuged for 3 min. Supernatant was saved and 400  $\mu$ l 50% (v/v) ethanol was added to the pellet and re-extracted using the same method. This was repeated again three times using 200  $\mu$ l 80% (v/v) ethanol. All supernatants were pooled together, and ethanol was added to give 2 ml.

To determine the sugar concentrations, 20  $\mu$ l aliquots were pipetted into 96-well plates and mixed with 160  $\mu$ l enzyme mixture containing activated 1.12 units glucose-6-phosphate dehydrogenase (Roche diagnostics, Switzerland), 0.25  $\mu$ mol NADP (Roche diagnostics, Switzerland), 0.5  $\mu$ mol ATP (Merck, Germany), and 0.75  $\mu$ mol Mg<sup>2+</sup> and measured at 340 nm by using a microplate reader (Synergy™ 2 Multi-Mode, BioTek, USA) at room temperature. When the extinction curve reached the plateau, 3.6 units hexokinase (Roche diagnostics, Switzerland) was added. The same step was repeated until 1 unit phosphoglucose isomerase (Roche diagnostics, Switzerland) and 20 units invertase (Roche diagnostics, Switzerland) were 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.

**2.5. Determination of Carotenoids.** Carotenoids were consecutively extracted by using 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  $\mu$ l precooled acetone (VWR, USA). After centrifuging at 13,200 rpm for 15 min, the supernatant was carefully filtered through a 0.2  $\mu$ m syringe filters (Sigma-Aldrich, USA). The filtered solvent was divided into two 1.5 ml Eppendorf tubes to which 500  $\mu$ l water and 300  $\mu$ l ethyl acetate were added. The mixture was then vortexed for 10 sec and centrifuged for 15 min at 13,200 rpm. 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, and column oven) coupled to an Agilent photodiode array (PDA) detector (Agilent Technologies, USA). 20  $\mu$ l standard and sample extract, respectively, was injected in the HPLC system. The carotenoids were separated on a ProntoSIL 200-3-C30 column (250  $\times$  4.6 mm; 3  $\mu$ m particle size) and a corresponding guard column (10  $\times$  4.0 mm; 3  $\mu$ m particle size) 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  $\mu$ l/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  $\beta$ -carotene, respectively. Quantification was done by external calibration with corresponding standards (DHI, Denmark). The concentrations were converted into  $\mu$ g/g fresh weight and averaged.

**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  $^{13}\text{C}_4$  malic acid was chosen.  $^{13}\text{C}_4$  malic acid (Sigma-Aldrich, USA) was considered due to the similarity of chemical structure with other organic acids. 1200  $\mu$ l 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  $^{13}\text{C}_4$  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 13,200 rpm. The supernatant was then filtered through a 0.2  $\mu$ m filter and diluted to the appropriate concentrations.

Quantification of organic acids was carried out using a Waters ACQUITY® UHPLC system (binary pump and autosampler) coupled to a Waters Xevo TQ-S® triple-quadrupole mass spectrometer (Waters Technologies Corp., MA, USA). Separation of three organic acids was achieved on a Nucleodur C18 Gravity-SB column (150  $\times$  3 mm, 3  $\mu$ m; Macherey-Nagel, Germany). The column was equipped with a precolumn (Macherey-Nagel, 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 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 was set 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 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.

**2.7. Determination of Carbon, Nitrogen, Sulfur, and Other Elements.** The ground dried sample was mixed with  $\text{HNO}_3$ ,  $\text{H}_2\text{O}_2$ , and HF, integrated by microwave. Then ICP-OES (inductively coupled plasma with optical emission spectroscopy, Elan 6000, PerkinElmer, 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  $\text{CO}_2$  and  $\text{SO}_2$  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).

**2.8. 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 2<sup>nd</sup> and 3<sup>rd</sup> clusters and three positions within one cluster were averaged. Quality analysis data from each season and each cultivar subjected to student's *t*-test were used. Experimental results were expressed as the mean  $\pm$  standard deviation.

### 3. Results and Discussion

**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 to 97.08  $\mu\text{mol}/\text{m}^2\text{s}$  and from 19.84 to 29.17°C, respectively (Figure 1). The values were higher in the summer of 2018, indicating 53.21 to 125.75  $\mu\text{mol}/\text{m}^2\text{s}$  and 23.49 to 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  $\text{mol}/\text{m}^2\text{d}$  [48], 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 [49]. Air temperature above 32°C during the day caused reduction of pollen formation and viability [50, 51]. From this, it could be assumed that plant growth was affected by extreme



TABLE 1: MRM parameters for organic acids.

| Compound                     | Precursor ion ( $m/z$ ) | Product ion ( $m/z$ ) | Cone voltage (V) | Collision energy (V) |
|------------------------------|-------------------------|-----------------------|------------------|----------------------|
| Malic acid                   | 132.9                   | 71.0                  | 22               | 16                   |
|                              | 132.9                   | 114.95                | 22               | 12                   |
| $^{13}\text{C}_4$ malic acid | 137.0                   | 119.0                 | 28               | 10                   |
| Ascorbic acid                | 175.02                  | 87.0                  | 24               | 16                   |
|                              | 175.02                  | 115.0                 | 24               | 12                   |
| Dehydroascorbic acid         | 173.0                   | 71.0                  | 24               | 16                   |
|                              | 191.02                  | 87.0                  | 32               | 16                   |
| Citric acid                  | 191.02                  | 111.0                 | 32               | 12                   |

The 1<sup>st</sup> row of malic, ascorbic, and citric acid is the quantifier mass transition, and the 2<sup>nd</sup> row is the qualifier mass transition used for quantification and compound confirmation, respectively.

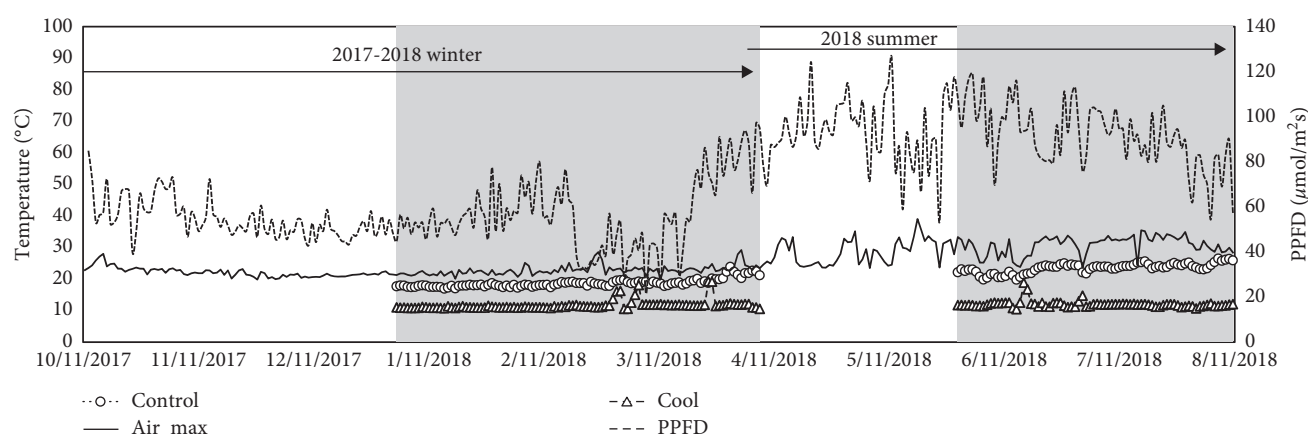


FIGURE 1: Moving average of daily maximum air temperature (Air\_max), photosynthetic photon flux density (PPFD) recorded inside the greenhouse from 11 October 2017 to 20 August 2018. The two seasons were indicated by arrows at the top of the graph. Root temperature treatment in the two seasons were indicated as gray, 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.

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).

### 3.2. Influence of Root Cooling on Plant Growth and Fruit Yield.

As indicated in Table 2, total yield and marketable yield per plant (the 2<sup>nd</sup> to 5<sup>th</sup> 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. [52], 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 [53, 54]. Reduction in water uptake leads to the stomata closure in order to maintain positive turgor pressure within the plant. The resultant CO<sub>2</sub> uptake and net photosynthetic rate became reduced, with eventual restriction of carbon production [55]. By contrast, Fujimura et al. [52] observed that the photosynthesis rate and stomatal conductance of tomato plant were not significantly affected by root chilling at 12°C

and suggested that the tomato plants showed acclimation within one week. Yan et al. [56] and Zhang et al. [57] suggested that loss of root cell viability and membrane lipid peroxidation inhibited plant growth. In our studies, the reduction of water content in the fruits (Table 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 [20, 58], which are primarily synthesized in root apical meristems [59]. 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 [60, 61].

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), 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 1). These results are similar to those reported by Adams et al. [50] and Domínguez et al. [51], which mentioned that air temperature above 32°C

TABLE 2: Effects of root cooling on yield and fruit and shoot growth parameters of cocktail tomato plants in the two seasons.

|                     |          | Fruit                     |                           |                          |                    |                        | Shoot                         |                           |                      |                |                  |
|---------------------|----------|---------------------------|---------------------------|--------------------------|--------------------|------------------------|-------------------------------|---------------------------|----------------------|----------------|------------------|
|                     |          | Total yield/<br>plant (g) | Marketable<br>yield/plant | Marketable/<br>total (%) | Mean<br>weight (g) | Equatorial<br>diameter | Longitudinal<br>diameter (mm) | Number of<br>fruits/plant | Water<br>content (%) | Height<br>(cm) | Diameter<br>(mm) |
| 2017-2018<br>winter | 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       |
|                     | Cool     | 1762.1 ± 109.1            | 1748.6 ± 116.7            | 99.2                     | 33.2 ± 2.7         | 39.7 ± 1.7             | 32.6 ± 0.7                    | 52.8 ± 2.4                | 94.34 ± 0.44         | 190.0 ± 8.0    | 15.0 ± 1.0       |
|                     | <i>p</i> | <b>0.047</b>              | <b>0.049</b>              |                          | 0.107              | 0.149                  | 0.213                         | 0.774                     | 0.270                | 0.357          | 0.145            |
|                     | 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       |
| 2018<br>summer      | 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       |
|                     | <i>p</i> | <b>0.018</b>              | <b>0.011</b>              |                          | 0.264              | 0.233                  | 0.238                         | 0.300                     | <b>0.010</b>         | 0.966          | 0.721            |
|                     | 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       |
|                     | 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       |
| 2018<br>summer      | <i>p</i> | 0.135                     | 0.272                     |                          | 0.721              | 0.823                  | 0.643                         | <b>0.018</b>              | 0.646                | <b>0.019</b>   | 0.186            |
|                     | 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         | 93.31 ± 0.71   | 93.31 ± 0.71     |
|                     | 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       |
|                     | <i>p</i> | 0.839                     | 0.640                     |                          | 0.991              | 0.920                  | 0.662                         | 0.748                     | 0.188                | 0.341          | <b>0.013</b>     |

Significant differences ( $p \leq 0.05$ ) are indicated in bold.

caused a reduction of pollen formation and viability. Thus, fruits of the 4<sup>th</sup> and 5<sup>th</sup> 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 1). Díaz-Pérez et al. [62] 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) indicated that root cooling to some extent relieved heat stress from aboveground for “Amoroso” in our experiment. Similarly, the fruit number and weight of cucumber were increased with root-zone cooling at high ambient temperature [63]. Furthermore, Mohammud et al. [64] as well as Kii and Araki [65] 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 and given lighting conditions as recently reviewed by Kawasaki and Yoneda [39].

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 1. The effect of root cooling was also dependent on the period of treatment. Wan et al. [55] observed a sharp reduction of water uptake and stomatal conductance at 24 hrs after 5°C root temperature treatment. Fennell and Markhart [66] 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.

### 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, accounts for nearly half of the total dry matter of tomato fruits, hence influencing tomato fruit quality largely [31]. The levels of glucose and fructose in control group ranged from 17.6 to 20.7 mg/g FW and 13.9 to 18.9 mg/g FW. These values were consistent with other studies, reporting ranges from 6.8 to 17.9 g/kg FW in tomato of different sizes [67, 68]. The levels of sucrose in control samples (ranging from 0.25 to 1.92 mg/g FW) were higher compared with previous report where sucrose is less than 0.05% of fresh weight [69]. The low levels of sucrose could be due to degradation of sucrose to glucose after transportation from the phloem [70]. In winter,

glucose and fructose levels were strongly influenced by root temperature (Table 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 3). Soluble sugars are important signaling molecules to regulate carbohydrates metabolism when plants are exposed to abiotic stress [71]. When exposed to low-temperature stress, soluble sugars have multiple roles, such as function as nutrients, as osmoprotectants to keep the turgor pressure, interacting with lipid layer to protect cellular membranes, as primary messengers in signal transduction, or as scavengers for hydroxyl radicals to mention some of the reported roles [72–74]. Another explanation is that, the reduction in sink strength of roots when exposed to suboptimal temperature without down-regulation of net photosynthesis [75]. 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. [52] denied this cause and attributed it to an excess of photoassimilates lead by the imbalance of sink and source capacity to suboptimal temperature.

Citric acid and malic acid are the major nonvolatile organic acids, responsible for the sourness in tomato fruits [76]. And the total organic acids were generally positively related to total acidity [77]. 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 those of malic acid (0.22–1.21 mg/g FW) [77, 78]. 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. [52]; the concentration of malic acid and citric acid was not influenced by root cooling regardless of the cultivar or the season. Shaw [79] 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 contrary, strawberries planted in summer contained higher levels of titratable acidity than in winter [80]. Lobit et al. [81] 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 and Camp [82] 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 [83]. The ascorbic

TABLE 3: Effects of root cooling on sugar, organic acid, and carotenoid concentrations of cocktail tomato fruits.

|                     |           |          | Sugars (mg/g FW) |                  |                 | Organic acids (mg/g FW) |                 |                 | Carotenoids ( $\mu\text{g/g}$ FW) |                   |
|---------------------|-----------|----------|------------------|------------------|-----------------|-------------------------|-----------------|-----------------|-----------------------------------|-------------------|
|                     |           |          | Glucose          | Fructose         | Sucrose         | Malic acid              | Citric acid     | Ascorbic acid   | Lycopene                          | $\beta$ -carotene |
| 2017-2018<br>winter | "Amoroso" | Control  | 18.91 $\pm$ 0.77 | 15.33 $\pm$ 0.79 | 1.32 $\pm$ 0.34 | 1.08 $\pm$ 0.11         | 5 $\pm$ 0.62    | 0.23 $\pm$ 0.01 | 72.48 $\pm$ 5.79                  | 7.51 $\pm$ 0.36   |
|                     |           | Cool     | 20.38 $\pm$ 0.95 | 16.47 $\pm$ 0.68 | 1.53 $\pm$ 0.21 | 1.09 $\pm$ 0.1          | 5.15 $\pm$ 0.31 | 0.22 $\pm$ 0.02 | 82.25 $\pm$ 10.67                 | 8.28 $\pm$ 0.71   |
|                     |           | <i>p</i> | <b>0.015</b>     | <b>0.024</b>     | 0.228           | 0.820                   | 0.611           | 0.734           | 0.086                             | <b>0.047</b>      |
|                     | "Delioso" | Control  | 19.08 $\pm$ 1.01 | 15.63 $\pm$ 1.23 | 0.91 $\pm$ 0.29 | 1.04 $\pm$ 0.12         | 4.75 $\pm$ 0.65 | 0.19 $\pm$ 0.01 | 85.08 $\pm$ 5.85                  | 7.60 $\pm$ 0.62   |
|                     |           | Cool     | 21.04 $\pm$ 1.17 | 17.72 $\pm$ 0.68 | 1.28 $\pm$ 0.12 | 0.99 $\pm$ 0.20         | 4.89 $\pm$ 0.61 | 0.23 $\pm$ 0.01 | 102.5 $\pm$ 5.63                  | 8.43 $\pm$ 0.54   |
|                     |           | <i>p</i> | <b>0.045</b>     | <b>0.033</b>     | 0.070           | 0.627                   | 0.710           | <b>0.001</b>    | <b>0.002</b>                      | 0.055             |
| 2018<br>summer      | "Amoroso" | Control  | 19.44 $\pm$ 1.03 | 17.49 $\pm$ 0.90 | 0.59 $\pm$ 0.19 | 0.29 $\pm$ 0.04         | 4.22 $\pm$ 0.28 | 0.23 $\pm$ 0.01 | 79.55 $\pm$ 6.90                  | 9.76 $\pm$ 0.64   |
|                     |           | Cool     | 20.78 $\pm$ 0.94 | 17.99 $\pm$ 0.76 | 0.71 $\pm$ 0.11 | 0.27 $\pm$ 0.06         | 4.28 $\pm$ 0.55 | 0.25 $\pm$ 0.02 | 72.52 $\pm$ 5.95                  | 9.45 $\pm$ 0.63   |
|                     |           | <i>p</i> | <b>0.041</b>     | 0.327            | 0.242           | 0.619                   | 0.821           | <b>0.019</b>    | 0.088                             | 0.411             |
|                     | "Delioso" | Control  | 18.41 $\pm$ 0.74 | 16.85 $\pm$ 0.66 | 0.99 $\pm$ 0.28 | 0.26 $\pm$ 0.02         | 3.60 $\pm$ 0.13 | 0.20 $\pm$ 0.01 | 79.72 $\pm$ 2.25                  | 10.66 $\pm$ 0.74  |
|                     |           | Cool     | 19.82 $\pm$ 0.70 | 17.70 $\pm$ 0.64 | 1.24 $\pm$ 0.23 | 0.26 $\pm$ 0.04         | 3.91 $\pm$ 0.38 | 0.24 $\pm$ 0.02 | 93.17 $\pm$ 3.08                  | 10.22 $\pm$ 1.23  |
|                     |           | <i>p</i> | <b>0.015</b>     | 0.071            | 0.16            | 0.700                   | 0.105           | <b>0.003</b>    | <b><math>\leq 0.001</math></b>    | 0.525             |

Significant differences ( $p \leq 0.05$ ) are indicated in bold.

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 [33]. Nonetheless, both cultivars contained slightly higher concentrations in summer than those harvested in winter, which is in accordance with Massot et al. [84] and Roselló et al. [85]. 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 [86, 87].

Amongst the carotenoids, only lycopene and  $\beta$ -carotene were measured in our studies. Lycopene predominates 60–74% of the carotenoids and is responsible for the red color [88]. The lycopene values reported in the present study (ranging from 71.3 to 108.9  $\mu\text{g/g}$  FW) were similar to early studies (ranging from 15 to 160  $\mu\text{g/g}$  FW) depending on different cultivars, ripeness, environmental factor, agricultural practices and postharvest storage conditions [89–92]. 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 fruit stage, synthesis of lycopene was inhibited [34, 85]. 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 [93, 94]. Krumbein et al. [34] 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

the 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 comprehensive effect of air temperature, root-zone temperature, solar irradiation, and other climatic factors.

$\beta$ -carotene, a precursor of vitamin A, is associated with the orange color in tomatoes [88]. The levels of  $\beta$ -carotene in control samples (ranging from 7.0 to 11.5  $\mu\text{g/g}$  FW) were lower than those reported by other authors in the range of 9.8–16.7 mg/kg FW [15]. In winter, the levels of  $\beta$ -carotene with root cooling were 10% higher than the control group in "Amoroso," while no differences were observed in "Delioso." In summer, the levels of  $\beta$ -carotene did not show differences in both cultivars when exposed to root cooling, but with higher levels than winter.  $\beta$ -carotene concentration was positively related to light intensity and less affected by high air temperature up to 38°C [95]. Therefore, higher light intensity accounted for the increase of  $\beta$ -carotene levels in summer. Lycopene and  $\beta$ -carotene indicated different sensitivities, and the response was dependent on the genotypes, which were consistent with Roselló et al. [85] and Gautier et al. [90].

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  $^1\text{O}_2$ ,  $\text{O}_2^-$ ,  $\text{H}_2\text{O}_2$ , and  $\cdot\text{OH}$  [96, 97]. To counteract the deleterious oxidative damage of ROS, plants have to produce antioxidant enzymes and antioxidants [17], 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 [98]; the concentration of vitamin C increased in strawberry [82]. 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 [32, 99]. Cucumber seedlings had higher sugar concentrations at 12°C than 20°C root temperature [56]. In two medicinal plants, *Catharanthus roseus* and *Nicotiana*



TABLE 4: Effects of root cooling on concentrations of cocktail tomato fruits in two seasons.

|            | 2017–2018 winter |               |           |                | 2018 summer    |              |               |               |              |               |               |              |
|------------|------------------|---------------|-----------|----------------|----------------|--------------|---------------|---------------|--------------|---------------|---------------|--------------|
|            | “Amoroso”        |               | “Delioso” |                | “Amoroso”      |              | “Delioso”     |               |              |               |               |              |
|            | Control          | Cool          | <i>p</i>  | Control        | Cool           | <i>p</i>     | Control       | Cool          | <i>p</i>     |               |               |              |
| 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  | <b>0.017</b> | 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    | <b>0.011</b> | 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    | <b>0.030</b> | 0.40 ± 0.03   | 0.34 ± 0.04   | <b>0.028</b> | 0.44 ± 0.03   | 0.40 ± 0.02   | <b>0.019</b> |
| S (%)      | 0.15 ± 0.01      | 0.15 ± 0.01   | 0.284     | 0.15 ± 0.00    | 0.13 ± 0.01    | <b>0.004</b> | 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   | <b>0.027</b> |
| Zn (mg/kg) | 1943 ± 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  | <b>0.016</b> | 21.68 ± 2.39  | 1811 ± 0.65   | <b>0.027</b> |
| Fe (mg/kg) | 53.10 ± 5.00     | 50.84 ± 8.24  | 0.582     | 53.4 ± 7.02    | 45.2 ± 4.60    | <b>0.042</b> | —             | —             | —            | —             | —             | —            |
| 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      | 547 ± 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 were below the detection limit.. Significant differences ( $p \leq 0.05$ ) are indicated in bold.

*tabacum*, the biosynthesis and accumulation of alkaloid were increased by altering root temperature during 48h root temperature treatment [100]. In agreement with previous studies, higher concentrations of glucose, fructose, ascorbic acid, lycopene, and  $\beta$ -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.

**3.4. Influence of Root Cooling on Concentrations of Elements.** Already Pollock and Eagles [101] reported that the effect of low temperature on carbon fixation and translocation is relatively mild, which was true in our case (Table 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 [102, 103]. By contrast, Wilson [104] and Hamblin et al. [105] 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 types of cultivars. 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 with reduced concentration in “Delioso” during winter, especially N, P, S, and Fe with varying reductions of 12.1%, 15.7%, 13.3%, and 15.4%, respectively (Table 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 [24] 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 [106]. Concentrations of ions in the fruits were influenced by root uptake, transport from root to shoot, and dilution effect caused by growth as well [107]. Considering the reduction in fresh biomass of fruits in our studies, the dilution effects by growth could be excluded.

In short-term, uptake of ions was severely affected by suboptimal root temperature which has been further proved in other species: grapevine [108], African snake tomato [38], spring barley [109], rice [110], and lettuce [111]. Altered root morphology under low root temperature was one reason [28, 102]. The mobility of cellular membrane phospholipids

was lost induced by low root temperature below the phase transition point [112]. Thus, ion carrier proteins and enzymes function were hampered [113, 114]. Another factor was related to limited energy available for ion uptake caused by reduced root respiration with root chilling [32], 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. [115] and Lee et al. [116]. However, tomato plants of our studies were exposed to low root temperature for extended periods. White et al. [45] and Engels et al. [117] 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 [118]), enhanced hydraulic conductivity [119], 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 [117]. In our setup, the isolation Matt covered and wrapped the shoot base; consequently, the base temperature was reduced as well. The uptake of N, P, K, S, Fe, and Zn did not demonstrate acclimation. On the contrary, the increased level of Mg in “Delioso” could be explained by the long-term adaption 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 complex processes: uptake rate of nutrients, retention of nutrients in root or for root growth, and transpiration rate [107]. Ca, P, and K in the leaf of snake gourd (*Trichosanthes cucumerina* L.) at suboptimal root temperature preferred to retain in the root than relocation to the shoot. Adebooye et al. [38] 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, and 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 [120, 121]. In our tomato fruits, the concentrations of Fe, Zn, and 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.

## 4. Conclusions

In conclusion, our results confirmed that it is possible to improve the fruit quality of cocktail tomato by modifying root temperature in winter and summer. Cooling root had a beneficial effect on the accumulation of sugars, especially glucose, in both cultivars and both seasons, despite a reduction of yield in winter. Though root temperature

differences between control and cooling treated group increased in summer, no reduction of yield was observed. Other than root temperature, the main differences between two seasons were the climatic conditions inside the greenhouse, which could explain the different impacts. Besides, the two cultivars behaved differently to root cooling. Root cooling improved the accumulation of vitamin C and lycopene in “Delioso” in both seasons, while only higher values of  $\beta$ -carotene in winter and vitamin C in summer were observed in “Amoroso.” Therefore, fruit quality can be improved as a result of application of root cooling during fruit growth and development, but the effects are also dependent on other climatic factors and cultivars.

### Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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