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# Differential Responses of Cherry Tomatoes (*Solanum lycopersicum*) to Long-Term Heat Stress

**Bo-Mi Park [1,†], Hyo-Bong Jeong [2,†], Eun-Young Yang [3], Min-Kyoung Kim [1], Ji-Won Kim [1], Wonbyoung Chae [1], Oak-Jin Lee [2], Sang Gyu Kim [2] and Sumin Kim [1,*]**

[1] Department of Environmental Horticulture and Landscape Architecture, Environmental Horticulture, Dankook University, Cheonan 31116, Republic of Korea
[2] Vegetable Research Division, National Institute of Horticultural & Herbal Science, RDA, Wanju 55365, Republic of Korea
[3] Department of Horticulture, Korea National College of Agriculture and Fisheries, Jeonju 54874, Republic of Korea
* Correspondence: sumin.kim@dankook.ac.kr; Tel.: +82-042-550-3644
† These authors contributed equally to this work.

**Abstract:** As global warming increases day/night temperatures as well as frequencies of heat waves, studying physiological responses in long-term heat stress is required for sustainable yield production in the future. In this study, effects of long-term heat stress on photosynthetic, morphological, and yield parameters of three cherry tomato accessions, HR17, HR22, and HR24, were evaluated. The experiment was conducted under two temperature greenhouse conditions, where temperature set-point for ventilation was 30 °C and 35 °C during the day for 57 days, respectively. Plants were harvested on the 35th days and 57th days after heat treatments, and their physiological and morphological characteristics and yield traits were measured. Under control conditions, HR17 and HR22 had 0.5–0.6 harvest index, while HR24 had 0.3 harvest index. On 35th days after heat treatment, although yield loss percentage of HR17 was high (43%), it produced the highest fruit yield among all three accessions. However, after longer heat treatment, HR24 produced the highest fruit yields among all accessions with the smallest yield loss (34%). Furthermore, yield loss was highly associated with reductions in nitrogen use efficiency and water content in plant body under heat stress. The results of this study will provide breeders with a new insight into selecting heat-tolerant genotypes in cherry tomatoes.

**Keywords:** harvest index; heat-tolerant genotype; greenhouse; nitrogen use efficiency; photosynthesis



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## 1. Introduction

Heat stress is caused by a combination of several environmental factors including temperature, relative humidity, air movement, solar radiation, and precipitation, with negative impacts on both plant growth and its productivity. In South Korea, the average air temperature has continuously increased since the early 21st century [1]. According to the report by Greenpeace [1], the number of extreme hot days has doubled over the past ten years. This heat warning has increasingly attracted the attention of many scientists and farmers, and several reports regarding heat stress in greenhouse vegetables were published [2,3]. Cherry tomato (*Solanum lycopersicum*) is an important vegetable, which is mostly grown in a greenhouse, with 30–40 million tons of average annual total production in South Korea [4]. The optimal day temperature for the growth of cherry tomato is in the range of 22 to 26 °C [5], and night optimal temperature is in the range of 15 to 20 °C [6]. Temperatures higher than the plants' optimal temperatures can cause heat stress, which negatively affects their growth, quality of fruits, and productivity.

Many previous studies have found that heat stress is highly related to reduction in the tomato fruit quantity or quality considerably caused by starch depletion [7], decrease in chlorophyll-carotenoid [8,9], abortion of the male gametophyte [10], decreases in root growth [11], etc. While immediate response or short-term heat stress response (<7 days) are relatively well studied [12–14], the physiological and photosynthetic processes underlying long-term (>45 days) heat stress in cherry tomato plants are still not well understood. Saidi et al. [15] reported that both short and long terms of heat exposures critically affected membrane transport and increased the damage to membrane fluidity and permeability of cells. In addition, long-term high temperature can have a greater negative impact on nutrient uptake. For example, the prolonged exposure to extreme heat can cause lower oxygen availability which leads to root browning [16,17]. As healthy portions of root turn brown, the plant may not absorb the nutrients as much as it needs, resulting in yield reduction. To have a better understanding on the effects of longer heat stress exposures, more experimentation evaluating physiological responses to long-term heat stress exposures is needed.

Heat stress responses, including physiological processes, growth and development, and yields, vary with cultivars [18] as well as species because they have different strategies to adapt the heat stress conditions [19]. Under stress conditions, plants either grow slowly to adapt stress conditions, or sacrifice their growth to respond to heat stress. The growth speed under stress is achieved through stress-triggered cell signaling [19]. The stress tolerance, also known as a relative ability to grow under stress condition, is usually determined by evaluating decreases in growth rate, fruit production, or biomass accumulation [19]. Some plants can increase the growth of certain plant organs, such as roots [20] or stems [21], as a response to stress exposures, which can result in higher biomass accumulation under stress. This higher biomass accumulation may reflect better stress tolerance. Although some plants can recover after short-term heat stress exposure (<1 day) [22], most plants have more sensitive stress-response programs under long-term heat stress. A comparison of various stress responses between different genotypes will help breeders to have a better understanding of different adaptation strategies or defense mechanisms, which will provide useful information for selection in breeding programs.

The main aim of this study is to investigate the heat stress responses of three different cherry tomato accessions that have different growth patterns and stress tolerance and are grown in a controlled greenhouse condition. Under heat stress, plants develop certain efficient strategies to avoid or tolerate the heat stress which allows them to adapt to and defend themselves from heat stress [23]. The adaptation strategies or stress resistances of three accessions were evaluated through the investigation of changes in physiological characteristics, yields, and nutrient uptake under long-term heat stress conditions.

## 2. Materials and Methods

### 2.1. Plant Material and Experimental Design

Three cherry tomato (*Solanum lycopersicum*) accessions, including HR17 (moderate heat-tolerant), HR22 (heat-sensitive), and HR24 (heat-tolerant commercial cultivar, 'Joeungyeo', Farm Hannong, Seoul, Republic of Korea), were compared. Both HR17 and HR22 have round shaped fruits, while HR24 has oval shaped fruits (Figure 1). The fruit weights for all cultivars ranged between 15 and 25 g.

Seeds of HR17 and HR22 were sown on 16 March 2022 in plastic trays (54 × 28 cm in size, 5 × 10 cm cells with pot volume 3.7 L) containing commercial bed soil ('Bio Sangto'; Seoul, Republic of Korea), containing cocopeat (67.5%), peat moss (17.0%), zeolite (5.0%), perlite (10.0%), pH adjuster (0.3%), humectant (0.014%), and fertilizers (0.185%) containing 270 mg kg$^{-1}$ of each of N, P, and K, respectively. The seedlings were grown to seven to nine fully expanded mature leaf stage (25–30 cm height) in a glasshouse (26 °C/18 °C in day/night (16/8 h) with relative humidity within 65–70%) at the National Institute of Horticultural and Herbal Science (Wanju, Republic of Korea, 35°83′ N, 127°03′ E). Tomato seedlings were transferred into greenhouses on 3 May 2022 (48 days after sowing). Black plastic mulch film was applied to the test beds. Plants were regularly watered with a drip irrigation system and fertigated weekly with nutrient solution A (N 5.5%, K 4.5%, Ca 4.5%, B 0.00014%, Fe 0.05%, Zn 0.0001%, and Mo 0.0002%) and B (N 6%, P 2%, K 4%, Mg 1%, B 0.05%, Mn 0.01%, Zn 0.005%, and Cu 0.0015%) (Mulpure, Daeyu, Seoul, Republic of Korea). Before heat treatment, the seedling plants were grown for two weeks in greenhouse conditions with a temperature set-point for ventilation of 30 °C during the day for adaptation in new environmental conditions. After two weeks, the temperature set-point of a greenhouse was reprogrammed to 35 °C during the day, while the other greenhouse for control was kept unchanged. The average air temperatures in a control greenhouse were relatively stable at 25–35 °C, and average air temperature for heat stress was 2–5 °C higher than that in the control greenhouse (Figure 2). The total number of days of heat treatments were shown in Figure 2. The number of days when the maximum temperatures were over 40 °C were 28 days and 10 days in a heat-treated and control greenhouse, respectively (Figure 2). The relative humidity was kept between 50 and 85% in both greenhouses.


**Figure 1.** Fruit phenotypes of HR17, HR22, and HR24 grown in control-condition greenhouse on 57th day after treatment.

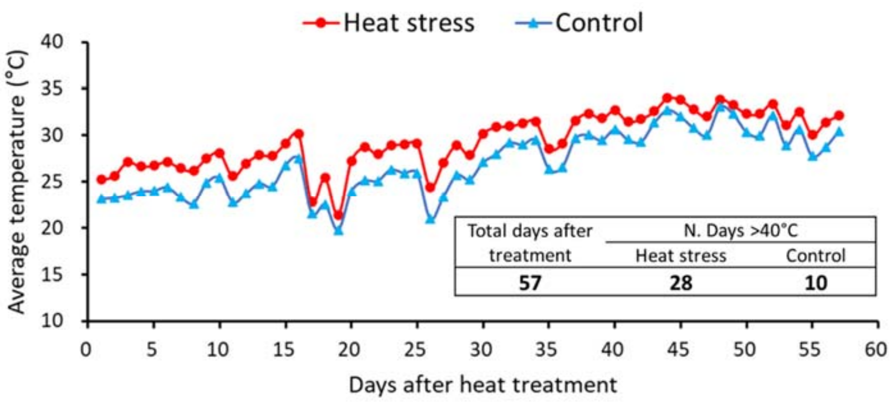


**Figure 2.** Average temperatures changes in heat-treated greenhouse and control greenhouse (Wanju, Republic of Korea). In table, the number of total treatment days, and the number of days when the daily maximum temperatures were over 40 °C in both greenhouses were presented. The heat stress was exposed to plants when plants were grown for about 62 days after sowing.

The experiment plots were laid out as a split design with three replicates. The main plot was a temperature treatment (heat stress or control), while the sub-plot was genotype in three accessions, including HR17, HR22, and HR24. The main plots were carried in two greenhouses. In each greenhouse, the sub-plot was laid out in randomized completed design with 1.5 m long single row plots consisting of five transplants (30 cm apart) and three replicates. The distance between single-row plots was 140 cm.

### 2.2. Evaluation of Physiological Characteristics and Yields

Plant height and stem thickness of three tomato cultivars were measured 1, 2, 4, 12, 25, 35, 43, and 57 days after heat treatment. For each replicate, at least three plants were measured. The plant height (cm) was measured from the base of plant to the tip. The plant stem thickness (mm) was measured using a digital caliper (CD-20APX, Mitutoyo Co., Ltd., Kanagawa, Japan). Plants were harvested on the 35th days and 57th days after heat treatment. At harvest, fresh weights (g) of fruits, leaves, and stems, and total leaf area (LA) were measured. The leaf area of each plant per $m^2$ covered by the crop was measured using an integrator of LA (LICOR-300, Lincoln, NE, USA). The harvested samples were dried at 70 °C, and dry weights (g) of stem, leaves, and fruits were measured. The moisture contents of each plant were measured using fresh and dry weights. The dry matter distribution was calculated using the dry weight data for each plant part.

### 2.3. Leaf Gas Exchange and Chlorophyll Fluorescence

On days of 1, 2, 4, 12, 25, 35, 43, and 57 days after heat treatment, a LI-COR LI-6800 (LI-COR Inc., Lincoln, NE, USA) gas exchange instrument was used to measure a net photosynthesis rate (A, µmol $m^{-2}s^{-1}$). On the 35th day, the readings were not stable; thus, the data from the 35th day were excluded from the data analysis. The measurements were taken on a newly fully expanded leaf between 10:00 and 14:00. The temperatures in Li-Cor chamber were set at 25 °C and 35 °C for control and heat treatment, respectively. The light intensity was 600 µmol $m^{-2}s^{-1}$, and the $CO_2$ concentration was set to 400 µmol $mol^{-1}$ $CO_2$ with 60% relative humidity for both greenhouse conditions. We exposed the selected leaves to various levels of irradiation for 4–5 min until the $CO_2$ uptake curve was stabilized, and then data were collected.

On the same days as the measurements of net photosynthesis rates, chlorophyll fluorescence was also collected on the same days as gas exchange measurements. Photon system Instrument (FluorPen, FFP 110, PSI, Drasov, Czech Republic) was used to measure PSII photochemical efficiency (Qy, Fv/Fm). The measurements were taken on a newly fully expanded leaf after 15 min of dark adaptation. After dark adaptation, saturating light was given at 3000 µmol (photon) $m^{-2}s^{-1}$, actinic light 1000 µmol (photon) $m^{-2}s^{-1}$, and measuring at 3000 µmol (photon) $m^{-2}s^{-1}$.

### 2.4. Determination of Electrolyte Leakage Potential in Seedlings Leaves under Heat Stress

A newly fully expanded leaf for each replicate was collected on days 2, 4, 12, 25, 35, 43, and 57 after heat treatment to measure electrolyte leakage. Leaves were sampled from three different plants for each accession by using a cork bore as a punch. The punched leaf disks were 5.5 mm in diameter. The punched samples were placed in a 15 mL tube containing 10 mL of deionized water and then incubated on a shaker at 25 °C for 30 min. The conductivity (EC1) of water was measured using a STARA-HB conductivity meter (Thermo Orion, Waltham, MA, USA). The tube was heated in a boiling water bath (100 °C) for 20 min and cooled at room temperature for 20 min, and then the conductivity (EC2) was measured. Final EC content was calculated as the percentage of EC1/EC2.

### 2.5. Calculation Nitrogen Use Efficiency

After harvesting plants on the 30th and 58th days after heat treatment, plants were dried and ground for nitrogen analysis. The total N contents in the dry matters of the fruits, stems, and leaves were analyzed based on the Kjedahl method (PanReacAplliChem, 2018) [24]. Approximately 1 g of each ground sample was placed into 300 mL glass tubes. The samples were digested in 15 mL concentrated $H_2SO_4$ using the Kjeldahl digestion system (SH420F, Hanon, Jinan, China). The digested samples were distilled with a small amount of NaOH using the distillation (K9840, Shandong Haineng Technology Instrument Co., Ltd., Shandong, China). After distillation, 0.1 N HCl was slowly added to the samples to determine total N contents. A more detailed protocol can be found in reports from

PanReacApliChem [24]. To find the total N (g/kg), it can be calculated with the following equation [24]:

$$\frac{\left(\text{ml HCl}_{\text{sample}} - \text{ml HCl}_{\text{bland}}\right) \times [\text{HCl}_{\text{con}}] \times 14.01 \times 100)}{1000 \times \text{weight of samples(g)}} \quad (1)$$

Nitrogen use efficiencies (NUE) of fruit (f) and biomass (b) for all varieties grown in both control and heat treatment greenhouses were calculated with the following equation:

$$\text{NUE}_{\text{f}}(\%) = \frac{\text{Total N yield in fruit}}{\text{Total N accumulation (soil + fertilizer)}} \times 100 \quad (2)$$

$$\text{NUE}_{\text{b}}(\%) = \frac{\text{Total N yield in biomass(stem + leaves)}}{\text{Total N accumulation (soil + fertilizer)}} \times 100 \quad (3)$$

### *2.6. Statistical Analysis*

The effects of heat treatment and accession were accessed by means of analysis of variance (ANOVA). The heat treatment and accession were treated as fixed factors. Pearson correlation procedures were conducted to analyze the relationships between the measured traits in control and heat treatment greenhouses in two different heat stress periods. For plant height, net photosynthesis rate, photosynthesis efficiency, and leaf leakage rates were also statistically tested by means of ANOVA. Treatment, days, accession, and interactions between them were tested. The effects of treatment, accession, organ parts, and interactions between them on NUE were also statistically tested. All statistical analyses were performed using the SAS program (SAS 9.4, Cary, NC, USA).

## 3. Results

### *3.1. Physiological Characteristic Measurements and Analysis Simple Correlation Factors*

Physiological characteristics and yields of three different cherry tomato accessions, including HR17, HR22, and HR24, were determined during various periods of exposure to heat stress in both greenhouses. Fresh weights, fruit weight, and harvest index of all three accessions were summarized in Table 1. According to the results of statistical analysis (Table 2), fresh weights on the 35th days were significantly affected by heat treatment ($p$ = 0.0032). Under heat stress, the fruit yields of three accessions were reduced. Although there were no significant differences found on the 57th day, except for in HR24, all accessions experienced a yield reduction under heat stress. Fruit weights significantly differed by accession ($p$ = 0.004) as HR22 had the highest fruit yield among all accessions. There was a significant interaction between accession and treatment ($p$ = 0.04). Under heat stress, HR17 showed the highest fruit yield on the 35th day after treatment. Although there were no significant differences found on the 58th day after treatment, under heat stress, HR24 produced the highest fruit yields among accessions. There were large fruit yield reductions observed in HR17 and HR22. On the 35th day, harvest index significantly differed by accession ($p$ = 0.001) as HR24 had the lowest harvest index of 0.25. On the 58th day, the harvest index of HR24 was still lower than others, but its harvest index increased from 0.25 to 0.4 under control conditions. The harvest index of other accessions decreased from 0.6 to 0.5. The largest yield losses were observed in HR22, with 52–57% of total yield and fruit yield losses at 35 days of treatment and 58–67% yield losses at 57 days of treatment. Based on these results, significant yield losses in fresh weight and fruits were also observed in HR17 (Table 1), showing fruit losses of 43% and 64% on the 35th and 57th days, respectively; however, they showed better yield than HR22 (Table 1).

Under control conditions, in general, three accessions had different growth patterns. HR17 and HR22 typically had higher harvest index and produced larger fruit yields than biomass (stem + leaves) at both harvest dates (Table 1). On the 35th day, for example, the HR17 had the highest harvest index of 0.61, approximately 2.44-fold higher than HR24.

On the 57th day, HR17 still had the highest fruit yields among three accessions. HR24 had the smallest harvest index on both harvest dates, but its fresh weight was higher or the same as HR17 under control conditions. HR22 had a similar harvest index and yields to HR17 up to the 35th day, with the smallest fresh weights on the 57th day, but its harvest index was kept in the range 0.54–0.62. According to the yield data in a control greenhouse, HR17 and HR22 tended to produce more fruits than plant biomass, while HR24 produced more plant biomass than fruits at the early growth stage. The growth patterns of the three accessions were similar at elevated temperatures. However, significant negative effects of long-term heat stress were found in plant biomass and fruit yields of two accessions, HR17 and HR22. HR24 showed the most tolerance to heat stress among all accessions. At 35 days of treatment, heat stress had no effect on fruit yields (300 g) of HR24, compared to the control (293 g). HR24 had the smallest harvest index value compared to other accessions over two periods, but it produced the highest fruit yield compared to other accessions on the 57th day. These results indicated that the growth pattern of HR24, which produced more plant biomass than fruits, had less heat stress effects for longer periods than the accessions that had harvest index values of around 0.5–0.6.

**Table 1.** Effects of long-term heat treatments on fresh weight (g), fresh fruit weight (g), harvest index of three cherry tomato accessions, including HR17, HR22, and HR24, in heat treatment and control conditions during two different time periods (35 days and 57 days). Yield differences of total weight and fruits were calculated for each accession in each heat-treated period. n.a. data are not available.

| | Control | | | Heat Stress | | | Yield Difference (%) | |
|---|---|---|---|---|---|---|---|---|
| **Tomato Varieties** | **Total Fresh Weight (g)** | **Fresh Fruit Weight (g)** | **Harvest Index** | **Total Fresh Weight (g)** | **Fresh Fruit Weight (g)** | **Harvest Index** | **Total Weight** | **Fruit** |
| After 35 days | | | | | | | | |
| HR17 | 1123 ± 192 | 687 ± 129 | 0.61 | 640 ± 14 | 395 ± 57 | 0.62 | −43 | −43 |
| HR22 | 1325 ± n.a. | 800 ± n.a. | 0.6 | 640 ± 14 | 348 ± 25 | 0.55 | −52 | −57 |
| HR24 | 1193 ± 131 | 293 ± 74 | 0.25 | 893 ± 272 | 300 ± 7 | 0.36 | −25 | 2 |
| After 57 days | | | | | | | | |
| HR17 | 1594 ± 1071 | 1078 ± 721 | 0.50 | 742 ± 366 | 390 ± 227 | 0.50 | −53 | −64 |
| HR22 | 1453 ± 787 | 821 ± 463 | 0.56 | 617 ± 293 | 268 ± 118 | 0.44 | −58 | −67 |
| HR24 | 1559 ± 764 | 664 ± 299 | 0.43 | 1430 ± 97 | 438 ± 146 | 0.30 | −8 | −34 |

**Table 2.** ANOVA of the effects of heat treatment (Trt.), accession (AC), and their interactions (Trt*AC) on morphological characteristics at alpha = 0.05. n.s. data are not available.

| Heat Stress Days | Factors | Fresh Weight | Fruit Weight | Harvest Index | Moisture Content | Leaf Area Index | Height | Stem |
|---|---|---|---|---|---|---|---|---|
| 35 | Trt. | 0.0032 | 0.004 | n.s. | 0.0002 | n.s. | n.s. | n.s. |
| | AC | n.s. | 0.008 | 0.001 | 0.003 | 0.04 | 0.004 | n.s. |
| | Trt*AC | n.s. | 0.04 | n.s. | n.s. | n.s. | n.s. | n.s. |
| 57 | Trt. | n.s. | n.s. | 0.0005 | 0.04 | n.s. | n.s. | n.s. |
| | AC | n.s. | n.s. | 0.0004 | n.s. | n.s. | 0.08 | n.s. |
| | Trt*AC | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |

In addition to yield investigations, several physiological characteristics were investigated in three tomato accessions (Table 3). The moisture contents were affected by treatment ($p$ = 0.0002) and accessions ($p$ = 0.003) on the 35th day, while moisture content was only significantly impacted by treatment ($p$ = 0.04). In general, the moisture contents were significantly reduced under heat stress on both the 35th and 57th days. On the 35th day, HR22 had higher moisture content than others under control and heat stress, while HR24 had the lowest moisture content among all accessions. Leaf area index significantly differed by accession, as HR24 had the highest leaf area index (1.69) among all accessions on the 35th day. The plant height of all accessions increased as temperature increased; however, it significantly differed by accession. HR22 was the shortest (162–172 cm) among all accessions, while both HR17 and HR24 were around 180–196 cm. There were no significant effects found on stem thickness. As shown in Table 1, HR17 and HR22 had the most significant heat stress effects on yields, showing significant reductions in fruit yield and leaf area index of HR17 and HR22 in heat stress conditions (Table 1).

**Table 3.** Effects of long-term heat treatment on moisture content, leaf area index (LAI), plant height, and stem thickness of three cherry tomato accessions, in HR17, HR22, and HR24, grown in two different time periods, 35 days and 57 days, in heat-treated and control greenhouses. n.a. data are not available.

| | | 35 Days | | | | 57 Days | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Effect** | **Tomato Varieties** | **Moisture Content (%)** | **LAI** | **Plant Height (cm)** | **Stem Thickness (mm)** | **Moisture Content (%)** | **LAI** | **Plant Height (cm)** | **Stem Thickness (mm)** |
| Control | HR17 | 89 ± 1.1 | 0.87 ± 0.05 | 180 ± 4 | 14.05 ± 0.33 | 87 ± 3.6 | 1.82 ± 1.40 | 186 ± 17 | 14.89 ± 1.55 |
| | HR22 | 90 ± n.a. | 1.28 ± n.a. | 162 ± 10 | 13.95 ± 0.7 | 85 ± 4.6 | 1.62 ± 1.32 | 171 ± 50 | 14.54 ± 2.15 |
| | HR24 | 87 ± 0.1 | 1.69 ± 0.37 | 185 ± 5 | 14.72 ± 0.47 | 86 ± 4.3 | 2.69 ± 1.96 | 189 ± 21 | 15.91 ± 3.6 |
| Heat | HR17 | 86 ± 0.8 | 0.62 ± 0.32 | 189 ± 6 | 15.25 ± 1.80 | 78 ± 6.4 | 1.16 ± 0.63 | 198 ± 7 | 15.11 ± 1.69 |
| | HR22 | 87 ± 0.3 | 0.67 ± 0.09 | 166 ± 24 | 14.82 ± 1.47 | 73 ± 12.2 | 1.48 ± 0.30 | 173 ± 37 | 15.24 ± 1.88 |
| | HR24 | 83 ± 0.2 | 1.18 ± 0.54 | 188 ± 5 | 14.92 ± 0.86 | 84 ± 1.8 | 2.52 ± 0.19 | 190 ± 11 | 15.31 ± 2.33 |

Under control conditions at 35 days after treatment, there were significant positive correlations between fruit yields with harvest index and moisture content, while a significant positive correlation with harvest index was observed under the heat stress condition (Figure 3). At 57 days after treatment, fruit yield had significant positive correlations with total yield under control conditions. Under the heat stress condition, fruit yields had a significant negative correlation with stem thickness, while there were significant positive correlations with total weight and moisture content (Figure 3).

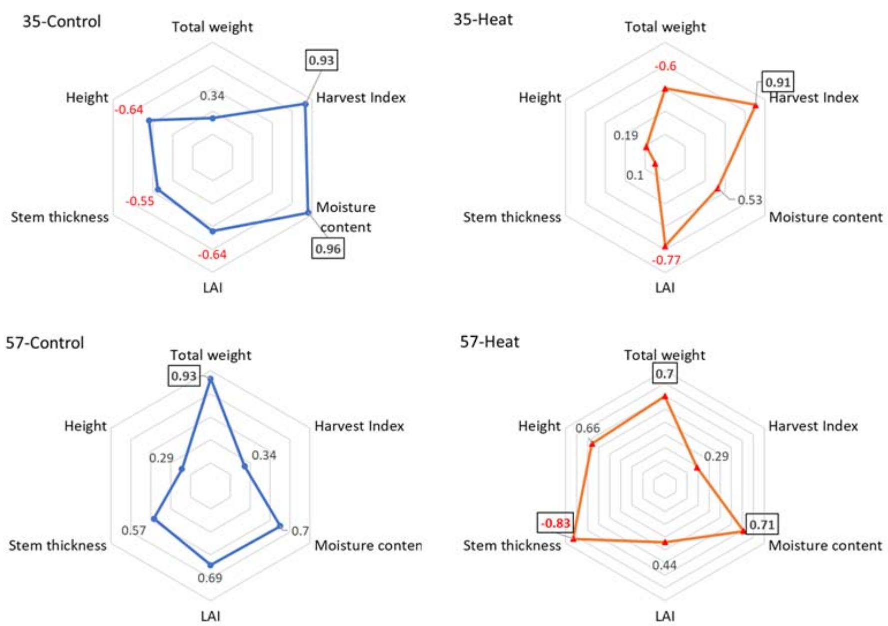


**Figure 3.** Correlation spider charts for showing correlation between fresh fruit weight and other morphological characteristics, including total weight, harvest index, moisture content, leaf area index (LAI), stem thickness, and plant height of all accessions treated with heat (orange line) for 35 days and 57 days and grown in control conditions (blue line). Red number indicates the negative relationship between accessions. Numbers in black box indicate significant correlation at alpha = 0.05.

### *3.2. Periodic Growth Responses in Morphological and Photoperiodic Parameters to Prolonged Heat Exposures*

Plant height, the PSII efficiency (Qy, Fv/Fm), and net photosynthesis rate (A) of three cherry tomato accessions were measured on days 2, 4, 12, 25, 35, 43, and 57 after heat treatment (Figure 4). According to statistical analysis, shown in Table 4, plant heights were significantly affected by heat treatment and heat exposure days, both at $p < 0.0001$. Additionally, there was a significant interaction between treatment and heat stress exposure days ($p < 0.0001$). In both HR17 and HR22, there was increased sensitivity to heat stress 5–10 days after treatments, coincident with decreased plant growth. Although there were no significant differences among accessions, HR24 maintained its maximum growth under long-term heat stress. Photosynthesis was significantly affected by interaction between treatment and exposure days at $p = 0.0049$ and $p = 0.006$, respectively. Under heat conditions, Qy values of HR22 continuously decreased after four days following treatment, while it started to decrease after 25 days under control conditions (Figure 4). HR17 showed slight decreases in Qy after 10 days in control conditions, while it maintained its photosynthesis efficiency under the heat stress condition. The HR24 maintained its photosynthesis efficiency under both control and heat stress conditions during the experiment periods. All accessions started to decrease their net photosynthesis rates (A) 25 days after treatments under heat stress conditions (Figure 4).

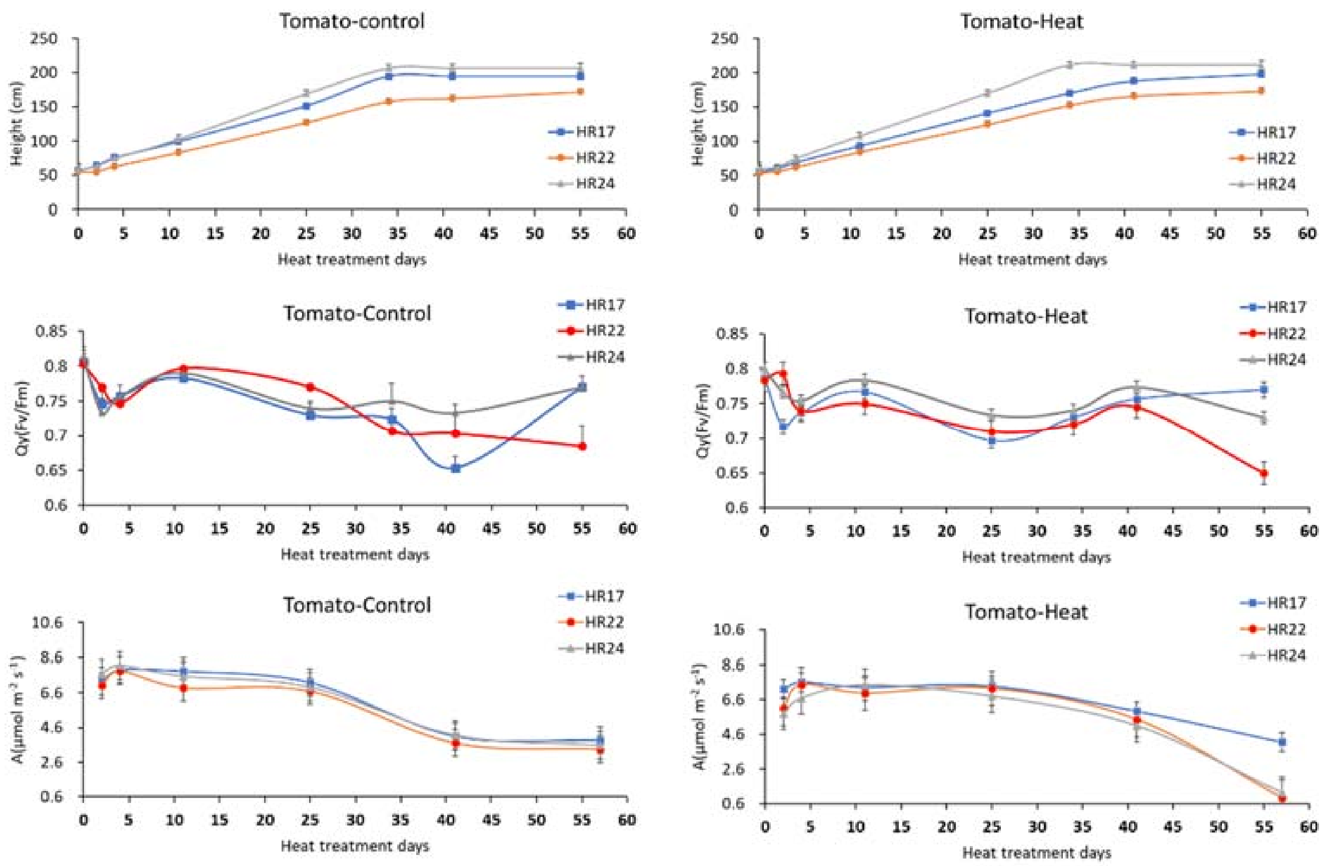


**Figure 4.** Effects of long-term heat treatment on plant height, the PSII efficiency (Qy, Fv/Fm), net photosynthesis rate (A) of three cherry tomato accessions, HR17, HR22, and HR24, grown in heat-treated and control greenhouses.

**Table 4.** ANOVA of effects of treatment days, heat stress, accessions, and their interactions on plant height, PSII efficiency, photosynthesis rates, and leaf heat damage levels. n.s. indicates no significant difference. * indicates the interaction between variables.

| Factors | Height | Qy | A | EC |
|---|---|---|---|---|
| Trt. Days (D) | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Treatment (Trt) | <0.0001 | n.s. | 0.058 | n.s. |
| D*Trt | <0.0001 | 0.0049 | 0.006 | 0.0345 |
| Accession (AC) | n.s. | n.s. | n.s. | n.s. |
| D*AC | n.s. | 0.0234 | n.s. | n.s. |
| Trt*AC | n.s. | n.s. | n.s. | n.s. |
| D*Trt*AC | n.s. | n.s. | n.s. | n.s. |

### *3.3. Effects of Long-Term Heat Stress on Leaf Heat Damage Levels and Nutrient Use Efficiency*

Leaf damage levels were investigated, with electrolyte leakages from leaf discs as an indicator of heat injury on days 2, 4, 12, 25, 35, 43, and 57 after heat treatment (Figure 5). According to statistical analysis, in Table 4, there was a significant interaction between growth days and treatment ($p = 0.0345$). Under control conditions, the injury was decreased as plants grew, while the leaf damage level tended to increase as heat stress exposure time increased under the heat stress condition (Figure 5). Although there was no significant difference detected among accessions, HR24 had the lowest damage level under the heat stress condition across the experimental periods (Figure 5), which reflected the NUE results. Although there were significant reductions in NUE for all accessions under the heat stress condition, NUE in HR24 vegetative organs was higher than other accessions at both 35 days (15.9%) and 57 days (18%) after treatments. Under control conditions, HR17 and HR22 had higher NUE in fruit organs at both 35 days and 57 days than HR24. However, all NUE in fruits at 57 days decreased to almost one-third of values at 35 days, under both control and heat stress conditions ($p < 0.0001$, Table 5).

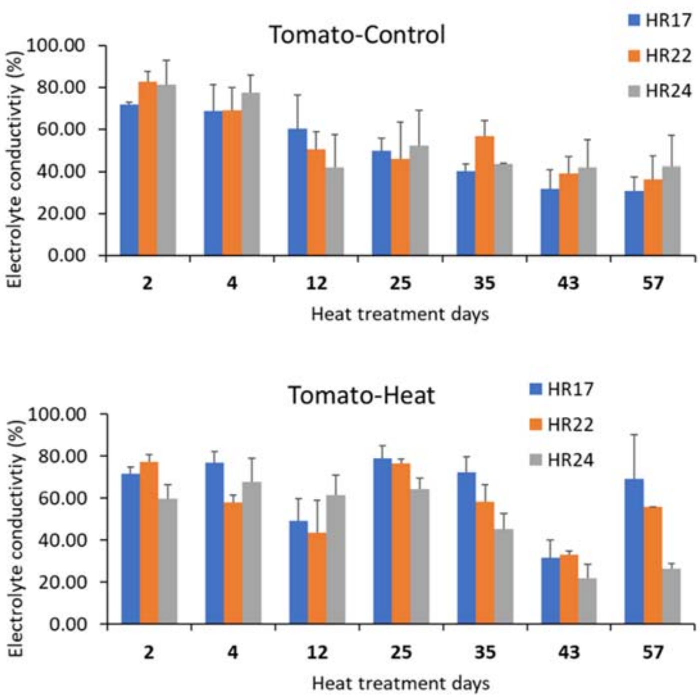


**Figure 5.** Effects of heat stress on leaf electrolyte leakage rate of three cherry tomato accessions for a long-term period in heat stress treatment and control greenhouses.

**Table 5.** Nitrogen Use Efficiency (NUE, %) and relative ratios of fruit and biomass NUE of three cherry tomato accessions grown in heat-treated and control greenhouses for 35 days and 57 days. ANOVA of effects of treatment days, heat stress, accessions, organs (biomass and fruit), and their interactions on NUE were presented. n.s. indicates no significant difference. * indicates the interaction between variables.

| Treatment | Accessions | NUE (%) | | | | Fruit/Biomass | |
|---|---|---|---|---|---|---|---|
| | | 35D | | 57D | | | |
| | | Fruit | Biomass | Fruit | Biomass | 35D | 57D |
| Control | HR17 | 49.2 | 49.9 | 14.1 | 33.1 | 1.0 | 0.4 |
| | HR22 | 40.7 | 40.6 | 13.3 | 26.9 | 1.0 | 0.5 |
| | HR24 | 32.2 | 75.9 | 10.1 | 53.1 | 0.4 | 0.2 |
| Heat | HR17 | 6.9 | 6.4 | 2.2 | 8.5 | 1.1 | 0.3 |
| | HR22 | 3.0 | 7.3 | 1.1 | 9.2 | 0.4 | 0.1 |
| | HR24 | 8.0 | 15.9 | 2.8 | 18.0 | 0.5 | 0.2 |

| Effect | *p*-value | Effect | *p*-value |
|---|---|---|---|
| Days | <0.0001 | Organ | <0.0001 |
| Treatment (Trt) | 0.045 | Days*Organ | <0.0001 |
| Days*Trt | 0.016 | Trt*Organ | n.s. |
| Accessions (AC) | <0.0001 | Days*Trt*Organ | 0.045 |
| Days*AC | n.s. | AC*Organ | <0.0001 |
| Trt*AC | n.s. | Days*AC*Organ | n.s. |
| Days*Trt*AC | n.s. | Trt*AC*Organ | n.s. |
| | | Days*Trt*AC*Organ | n.s. |

## 4. Discussion

The physiological and yield responses in heat stress conditions were significantly varied by accessions and growth stages. According to the previous studies [13,25], traits associated with heat stress can vary during the vegetative and reproductive growth stages. Thus, it is crucial to monitor changes in vegetative and reproductive traits from multiple genotypes during both vegetative and reproductive stages to adapt to elevated temperatures in the present and future [1]. In addition, the identification of key traits associated with heat tolerance will enhance the speed of the tomato breeding program by the early selection of heat-tolerant genotypes.

This study had monitored the growth, photosynthesis, and yield changes during different growth stages in three cherry tomato accessions. These tomato accessions had different growth patterns under control conditions. For example, HR17 (moderate heat-tolerant accession) and HR22 (heat-sensitive accession) tended to have high harvest index around 0.54–0.6, which means that it produced slightly more fruits than biomass under control conditions. In contrast, HR24 (heat-tolerant accession) produced less fruit yield compared to its biomass, resulting in lower harvest index values (0.25–0.46). As shown in Figure 2, the number of days when the maximum temperatures reached over 40 °C in a heat treatment greenhouse was 28 days, which was three times greater than in control conditions. As plants had exposure to more extreme heats for a long-term period, significant yield reductions were mostly observed in two accessions, HR17 and HR22. When plants produced more fruits in earlier stages, they were more sensitive to heat stress, which resulted in 43–67% fruit yield losses as in HR17 and HR22. When plants produced more biomass than fruits (e.g., HR24), they tended to be more tolerant to heat stress than others. Although HR24 produced less fruit yield than HR17 on the 34th day, its fruit yield was slightly increased by 2% under the heat stress condition. At the 57th day, the fruit yield of HR24 was only decreased by 34% and produced the highest fruit yields among all accessions.

Based on the results from correlation analysis, under heat stress conditions, fruit yields were still strongly influenced by harvest index up to the 35th day, but total fresh weight, including biomass and fruits, was strongly associated with fruit yields at the 57th day. These results indicate that plants with more leaves will be more tolerant to long-term heat stress. HR24 increased height and leaf area index as temperature increased, which resulted in the highest fresh total weight among all accessions under the heat stress condition. Since the leaf area index was high in HR24, its photosynthesis efficiency was maintained higher than other accessions for a long-term period. Many previous studies have reported that heat stress significantly affected vegetative parameters, including leaf fresh and dry weight, leaf area, plant height, stem thickness, etc. [26]. However, there are conflicting and contradictory results regarding correlation between heat stress tolerant ability and growth responses. In many cases, greater elongations of stem and leaves were observed as temperature increased [27]. This elongation response may help plants to avoid the heat dissipation through raising their leaves and meristematic tissues towards a cooling breeze [28]. In addition, heat-tolerant plants are characterized by high photosynthesis rates [29,30], and sustain gas exchange rate under heat stress [31]. Unlike our results, some previous studies had different results, showing that vegetative growth was not strongly associated with fruit yields [32,33]. Moreover, Abdelmageed and Gruda [34] reported that vegetative growth parameters, including fresh leaf weight and leaf area, of heat-tolerant tomato plants were smaller at a high temperature. This suggests that comparative studies among multiple heat-tolerant tomato accessions that have different growth patterns are needed to understand interactions between genotype and abiotic stress.

The results of NUE reflected the growth pattern of each accession. HR17 had high NUE in fruits at 35 days of growth under control conditions, and resulted in higher fruit yield production. In contrast, HR24 had high NUE in biomass because it produced more stem and leaves than fruit parts at 35 days of plant growth. Under heat stress, the overall NUE reduced significantly for all accessions. Many studies have reported that the heat stress reduced the NUE due to reduced photosynthetic leaf area [35–37] and root growth [11]. Under heat stress, fresh yield of all accessions was reduced, which resulted in a large reduction in NUE. HR24 had greater NUE than others because its fresh weight was only reduced by 34% under long-term heat stress. NUE is highly correlated with water use efficiency (WUE) [38]. According to Elio et al. [38], when cherry tomato WUE decreased, the plant absorbed less nitrogen, resulting in yield reduction. A similar result was also observed in this study. The moisture contents were significantly affected by treatment, as seen on the 35th and 57th days after treatment. Under heat stress, water contents were significantly reduced in comparison to control conditions. A greater damage level observed under heat stress also explained why NUE and water content decreased as temperature increased. A reduction in the number of healthy leaf cells caused water stress-induced leaf area reduction, which was also observed in many other species (e.g., *Sesbania aculeate*, *Phaseolus vulgaris* [39], *Sesamum indicum* [40], *Pennisctum glaucum* L. [41], and *Solanum lycopersicum* [42]).

## 5. Conclusions

In this study, the responses of three cherry tomato accessions, that have different growth patterns, to long-term heat stress were evaluated. The results of this study suggested some key characteristics that made the accessions more tolerant to long-term heat stress. For comparatively short-term heat stress, genotypes with a high harvest index (for example, HR17) were more favorable. However, for long-term heat stress, genotypes that had greater stem elongation and leaf area were more tolerant to heat stress. In addition, according to our results, NUE was correlated with water stress, and all accessions had lower NUE and water contents under heat stress. This suggests that a sufficient amount of water irrigation might help plants to survive under long-term heat stress. Further investigations of combination effects of water stress and heat stress on the growth of these accessions are needed to find the optimal cropping management under long-term heat stress conditions.

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## References

1. Greenpeace. Extreme Temperature Rise in East Asia Cities. 2021. Available online: https://www.greenpeace.org/eastasia/press/6727/greenpeace-maps-growing-climate-risk-from-extreme-weather-in-chinas-major-cities/ (accessed on 7 September 2022).
2. Nie, W.F.; Xing, E.; Wang, J.; Mao, Y.; Ding, X.; Guo, J. Emerging Strategies Mold Plasticity of Vegetable Plants in Response to High Temperature Stress. *Plants* **2022**, *11*, 959. [CrossRef]
3. Formisano, L.; El-Nakhel, C.; Corrado, G.; Pascale, S.; Rouphael, Y. Biochemical, Physiological, and Productive Response of Greenhouse Vegetables to Suboptimal Growth Environment Induced by Insect Nets. *Biology* **2020**, *9*, 432. [CrossRef]
4. Kostat. Tomato Reports. 2022. Available online: https://sri.kostat.go.kr/board.es?mid=a90102010100&bid=11918 (accessed on 7 September 2022).
5. Sato, S.; Peet, M.; Thomas, J. Physiological factors limit fruit set of tomato (*Lycopersicon esculentum* Mill.) under chronic, mild heat stress. *Plant Cell Environ.* **2000**, *23*, 719–726. [CrossRef]
6. Charles, W.B.; Harris, R. Tomato fruit-set at high and low temperatures. *Can. J. Plant Sci.* **1972**, *52*, 497–506. [CrossRef]
7. Dinar, M.; Rudich, J.; Zamski, E. Effects of heat stress on carbon transport from tomato leaves. *Ann. Bot.* **1983**, *51*, 97–103. [CrossRef]
8. Camejo, D.; Rodríguez, P.; Morales, M.A.; Dell'amico, J.M.; Torrecillas, A.; Alarcón, J.J. High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. *J. Plant Physiol.* **2005**, *162*, 281–289. [CrossRef]
9. Saeed, A.; Hayat, K.; Khan, A.A.; Iqbal, S. Heat tolerance studies in tomato (*Lycopersicon esculentum* Mill.). *Intl. J. Agr. Biol.* **2007**, *9*, 649–652.
10. Reddy, K.R.; Kakani, V.G. Screening Capsicum species of different origins for high temperature tolerance by in vitro pollen germination and pollen tube length. *Sci. Hortic.* **2007**, *112*, 130–135. [CrossRef]
11. Giri, A.; Heckathorn, S.; Mishra, S.; Krause, C. Heat Stress Decreases Levels of Nutrient-Uptake and-Assimilation Proteins in Tomato Roots. *Plants* **2017**, *6*, 6. [CrossRef]
12. Bita, C.E.; Zenoni, S.; Vriezen, W.H.; Mariani, C.; Pezzotti, M.; Gerats, T. Temperature stress differentially modulates transcription in meiotic anthers of heat-tolerant and heat-sensitive tomato plants. *BMC Genom.* **2011**, *12*, 384. [CrossRef]
13. Rajametov, S.N.; Yang, E.Y.; Jeong, H.B.; Cho, M.C.; Chae, S.Y.; Paudel, N. Heat Treatment in Two Tomato Cultivars: A Study of the Effect on Physiological and Growth Recovery. *Horticulturae* **2021**, *7*, 119. [CrossRef]
14. Qu, G.Q.; Liu, X.; Zhang, Y.L.; Yao, D.; Ma, Q.M.; Yang, M.Y.; Luo, Y.B. Evidence for programmed cell death and activation of specific caspa se-like enzymes in the tomato fruit heat stress response. *Planta* **2009**, *229*, 1269–1279. [CrossRef]
15. Saidi, Y.; Peter, M.; Finka, A.; Cicekli, C.; Vigh, L.; Goloubinoff, P. Membrane lipid composition affects plant heat sensing and modulates Ca2+-dependent heat shock response. *Plant Signal. Behav.* **2010**, *5*, 1530–1533. [CrossRef]
16. Fukuoka, N.; Enomoto, T. The occurrence of internal browning induced by high soil temperature treatment and its physiological function in Raphanus root. *Plant Sci.* **2001**, *161*, 117–124. [CrossRef]
17. Wells, C.E.; Eissenstat, D.M. Beyond the roots of young seedlings: The influence of age and order on fine root physiology. *J. Plant Growth Regul.* **2002**, *21*, 324–334. [CrossRef]
18. Guo, T.; Gull, S.; Ali, M.M.; Yousef, A.F.; Ercisli, S.; Kalaji, H.M.; Telesiński, A.; Auriga, A.; Wróbel, J.; Radwan, N.S.; et al. Heat stress mitigation in tomato (*Solanum lycopersicum* L.) through foliar application of gibberellic acid. *Sci. Rep.* **2022**, *12*, 11324. [CrossRef]
19. Zhang, H.; Zhao, Y.; Zhu, J.K. Thriving udner stress: How plants balance growht and the tresess resonse. *Dev. Cell.* **2020**, *55*, 529–543. [CrossRef]
20. Hsiao, T.C.; Acevedo, E.; Fereres, E.; Henderson, D.W. Water stress, growth and osmotic adjustment. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **1976**, *26*, 479–500. [CrossRef]
21. Zhao, Y.; Xing, L.; Wang, X.; Hou, Y.J.; Gao, J.; Wang, P.; Duan, C.G.; Zhu, X.; Zhu, J.K. The ABA receptor PYL8 promotes lateral root growth by enhancing MYB77-dependent transcription of auxin-responsive genes. *Sci. Signal.* **2014**, *3*, 53. [CrossRef]

22. Song, Y.; Chen, Q.; Ci, D.; Shao, X.; Zhang, D. Effects of high temperature on photosynthesis and related gene expression in poplar. *BMC Plant Biol.* **2014**, *14*, 111. [CrossRef]
23. Elangbam, L.D.; Sudhir, K.; Basanta, S.; Susheel, K.S. Adaptation Strategies and Defense Mechanisms of Plants during Environmental Stress. *Med. Plants* **2017**, *20*, 359–413. [CrossRef]
24. PanReacApliChem. Nitrogen Determination by Kjeldahl Method. 2018. Available online: https://byjus.com/chemistry/kjeldahl-method/ (accessed on 21 January 2023).
25. Sun, M.; Jiang, F.; Zhang, C.; Shen, M.; Wu, Z. A new comprehensive evaluation system for thermo-tolerance in tomato at different growth stage. *J. Agric. Sci. Technol. B* **2016**, *6*, 152–168. [CrossRef]
26. Aldubai, A.A.; Alsadon, A.A.; Migdadi, H.H.; Alghamdi, S.S.; Al-Faifi, S.A.; Afzal, M. Response of Tomato (*Solanum lycopersicum* L.) Genotypes to Heat Stress Using Morphological and Expression Study. *Plants* **2022**, *11*, 615. [CrossRef] [PubMed]
27. Myster, J.; Moe, R. Effect of diurnal temperature alterations on plant morphology in some greenhouse crops—A mini review. *Sci. Hort.* **1995**, *62*, 205–215. [CrossRef]
28. Gray, W.M.; Ostin, A.; Sandberg, G.; Romano, C.P.; Estelle, M. High temperature promotes auxin-mediated hypocotyl elongation in Arabidopsis. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 7197–7202. [CrossRef] [PubMed]
29. Scafaro, A.P.; Haynes, P.A.; Atwell, B.J. Physiological and molecular changes in Oryza meridionalis Ng, a heat-tolerant species of wild rice. *J. Exp. Bot.* **2010**, *61*, 191–202. [CrossRef]
30. Nagarajan, S.; Jagadish, S.V.K.; Prasad, A.H.; Thomar, A.K.; Anand, A.; Pal, M.; Agarwal, P.K. Local climate affects growth, yield and grain quality of aromatic and non-aromatic rice in northwestern India. *Agric. Ecosyst. Environ.* **2010**, *138*, 274–281. [CrossRef]
31. Craita, E.B.; Tom, G. Plant tolerance to high temperature in a changing environment: Scientific fundamentals and production of heat stress-tolerant crops. *Front. Plant Sci.* **2013**, *4*, 273. [CrossRef]
32. Zhou, R.; Yu, X.; Ottosen, C.O.; Rosenqvist, E.; Zhao, L.; Wang, Y.; Yu, W.; Zhao, T.; Wu, Z. Drought stress had a predominant effect over heat stress on three tomato cultivars subjected to combined stress. *BMC Plant Biol.* **2017**, *17*, 24. [CrossRef]
33. Xu, J.; Wolters-Arts, M.; Mariani, C.; Huber, H.; Rieu, I. Heat stress affects vegetative and reproductive performance and trait correlations in tomato (*Solanum lycopersicum*). *Euphytica* **2017**, *213*, 156. [CrossRef]
34. Abdelmageed, A.H.A.; Gruda, N. Influence of heat shock pretreatment on growth and development of tomatoes under controlled heat stress conditions. *J. Appl. Bot. Food Qual.* **2007**, *81*, 26–28. [CrossRef]
35. Farooq, M.; Bramley, H.; Palta, J.A.; Siddique, K.H.M. Heat stress in wheat during reproductive and grain-filling phases. *Crit. Rev. Plant Sci.* **2011**, *30*, 491–507. [CrossRef]
36. Mu, Q.; Cai, H.J.; Sun, S.K.; Wen, S.S.; Xu, J.T.; Dong, M.Q.; Saddique, Q. The physiological response of winter wheat under short-term drought conditions and the sensitivity of different indices to soil water changes. *Agric. Water Manag.* **2021**, *243*, 106475. [CrossRef]
37. Alsamir, M.; Mahmood, T.; Trethowan, R.; Ahmad, N. An overview of heat stress in tomato (*Solanum lycopersicum* L.). *Saudi J. Biol. Sci.* **2021**, *28*, 1654–1663. [CrossRef]
38. Elio, E.T.M.; Chao, W.; Ashutus, S.; Elke, B.; Peiman, Z.; Beata, B.K.; Aminu, D.; Yaosheng, W. Reduced nitrogen proportion during the vegetative growth stage improved fruit yield and nitrogen uptake of cherry tomato plants under sufficient soil water regime. *Acta Agric. Scand. B Soil Plant Sci.* **2022**, *72*, 700–708. [CrossRef]
39. Ashraf, M.; Iram, A.I. Drought stress induced changes in some organic substances in nodules and other plant parts of two potential legumes differing in salt tolerance. *Flora* **2005**, *200*, 535–546. [CrossRef]
40. Hassanzadeh, M.; Asghari, A.; Jamaati-E-Somarin, S.H.; Saeidi, M.; Zabihi-E-Mahmoodabad, R.; Hokmalipour, S. Effects of water deficit on drought tolerance indices of sesame (*Sesamum indicum* L.) genotypes in Moghan region. *J. Environ. Sci.* **2009**, *3*, 116–121. [CrossRef]
41. Kusaka, M.; Lalusin, A.G.; Fujimura, T. The maintenance of growth and turgor in pearl millet (*Pennisetum glaucum* [L.] Leeke) cultivars with different root structures and osmo-regulation under drought stress. *Plant Sci.* **2005**, *168*, 1–14. [CrossRef]
42. Medyouni, I.; Zouaoui, R.; Rubio, E.; Serino, S.; Ahmed, H.B.; Bertin, N. Effects of water deficit on leaves and fruit quality during the development period in tomato plant. *Food Sci. Nutr.* **2021**, *9*, 1949–1960. [CrossRef]