Bioactive Compounds of Tomato Fruit in Response to Salinity, Heat and Their Combination

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Abstract: In light of foreseen global climatic changes, we can expect crops to be subjected to several stresses that may occur at the same time, but information concerning the effect of long-term exposure to a combination of stresses on fruit yield and quality is scarce. This work looks at the effect of a long-term combination of salinity and high temperature stresses on tomato yield and fruit quality. Salinity decreased yield but had positive effects on fruit quality, increasing TSS, acidity, glucose, fructose and flavonols. High temperatures increased the vitamin C content but significantly decreased the concentration of some phenolic compounds (hydroxycinnamic acids and flavanones) and some carotenoids (phytoene, phytofluene and violaxanthin). An idiosyncrasy was observed in the effect of a combination of stresses on the content of homovanillic acid O-hexoside, lycopenes and lutein, being different than the effect of salinity or high temperature when applied separately. The effect of a combination of stresses may differ from the effects of a single stress, underlining the importance of studying how stress interactions may affect the yield and quality of crops. The results show the viability of exploiting abiotic stresses and their combination to obtain tomatoes with increased levels of health-promoting compounds.

Keywords: sugars; carotenoids; phenolic; antioxidants; nutritional quality; high temperature; NaCl

1. Introduction

Tomato (Solanum lycopersicum L.) is an important horticultural crop worldwide and one of the most consumed vegetables in the world. Several abiotic stresses, such as water deficit, salinity and extreme temperatures, can affect crop production. The effects of one of these single stresses on plant production and physiological, biochemical and molecular changes have been widely studied in the literature. In particular, tomato plants are often cultivated in arid or semi-arid regions of the world, where salinity and high temperature threaten to become, or already are, a problem. The effect of irrigation with saline waters on tomato fruit has been well documented, indicating a decrease in yield and changes in fruit quality [1], usually leading to better tasting fruits [2,3]. In relation to high temperatures, several studies have shown a decrease in tomato fruit yield [4,5], and some authors have indicated that secondary metabolites were more affected than primary metabolites [6]. Moreover, different responses to heat conditions amongst tomato genotypes have been associated with the different effects of heat on some photosynthetic parameters [7].
Agricultural land in arid or semi-arid regions can be affected not only by a single stress, but by several stress conditions simultaneously. Moreover, considering the predicted global climatic changes, we can expect this situation to be exacerbated, with serious consequences [8]. Recently, several studies have focused on the effect of combinations of various stresses on plant physiological responses [9,10]. Some results have indicated that when plants are subjected to a combination of abiotic stresses, the response may be different from that under each stress applied separately [10].

Similarly, results have indicated that the combination of various stresses had a greater impact on plant growth and productivity than a single stress [8,11]. Nevertheless, some reports have shown that a combination of stresses (e.g., salinity and heat) may lead to better plant behavior than when each stress was applied individually [9,12,13]. However, it is important to note that most of these studies reported on the short-term physiological effects of stress combinations, while information about the effect of long-term exposure to a combination of stresses on fruit yield and quality is scarce.

The aspects of productivity and sensory quality have attracted most attention, but recently, there has been increasing interest in the nutritional value of fruits and vegetables [14], as consumers demand products with a high content of health-promoting constituents. In this respect, tomato is an important source of carotenoids such as β-carotene, a precursor of vitamin A; lycopene, which has been associated with a reduction in the risk of cancer, cardiovascular disease and macular degeneration [15]; lutein, which plays a fundamental role in the protection of vision [16] and in preventing age-related maculopathy [17]; others that have been less well studied, such as phytoene and phytofluene, which may contribute to inhibiting the progression of atherosclerosis [18]. Furthermore, tomato is also a source of phenolic compounds such as flavonoids and hydroxycinnamic acid derivatives and vitamins such as ascorbic acid. All of these compounds contribute to its antioxidant properties and beneficial health effects [19].

The above-mentioned compounds are important for the commercial quality of tomato and can be affected by factors such as variety and environmental, agricultural and post-harvest conditions [20]. Moreover, using controlled abiotic stress may be an interesting approach to improve the nutraceutical value of fruits and vegetables [21]. Taking all of this into consideration, the aim of this work was to study the effect of a combination of different stresses (salinity and high temperature) on tomato yield and fruit quality. Unlike most previous studies found in the literature, this study involves the long-term exposure to the combination of stresses, according to the current growing conditions, and allows for elucidating the effect of these stresses on the final bioactive composition of the fruit.

2. Materials and Methods

The study was carried out from April (mid-spring) to July (mid-summer) in two polycarbonate greenhouses. Tomato seedlings (*Solanum lycopersicum* L.) were transplanted to 120 L containers (1 plant per container) with aerated Hoagland nutrient solution (pH = 5.5–6.1) prepared with osmosis-generated water and 1 mM NaCl in order to reach an optimum conductivity value (2.2 dS m\(^{-1}\)) for tomato plant and fruit development [22]. The cultivar used was Boludo, provided by Monsanto, which is an indeterminate hybrid variety for fresh consumption with a high fruit-setting capacity at high temperature and rounded fruits of medium size and homogeneous color at maturity. Thirteen days after transplanting (DAT), the temperature treatments were started, maintaining one of the greenhouses (greenhouse 1) at a maximum of 25 °C during the day, while in the other (greenhouse 2), the maximum temperature was gradually increased over three days to reach a final maximum temperature of 35 °C during the day. These temperatures were reached naturally (without any heat source). To avoid exceeding these temperatures, the greenhouses were fitted with a control system that included shade nets, zenithal windows and a cooling system (Munters, Madrid, Spain). The shade nets were activated simultaneously in both greenhouses for twelve hours per day starting at 8 a.m. In addition, zenithal windows were opened from 6 a.m. until the temperature exceeded a temperature value of 20 °C.
Thereafter, the maximum temperature set in each greenhouse was maintained using the cooling system. Night temperatures ranged between 20 and 13 °C throughout the growing period in a similar way in both greenhouses. The saline treatment (60 mM NaCl) was started (16 DAT) in half of the containers in each greenhouse, through the application of 20 mM NaCl for three consecutive days in order to avoid osmotic shock. The salinity level (60 mM NaCl, 7.8 dS m^{-1}) was selected on the basis of previous results, which showed that this level increased tomato fruit quality and reduced yield without drastically affecting plant development [22,23]. These combinations provided a total of four treatments: control (C, 25 °C + 1 mM NaCl), saline (S, 25 °C + 60 mM NaCl), heat (H, 35 °C + 1 mM NaCl) and heat + salinity (S + H, 35 °C + 60 mM NaCl), distributed in a completely randomized design with 6 replications (plants) per treatment (Figure 1). The nutrient solutions were analyzed every week, and nutrients were added when they were 10% below the starting level. The pH was adjusted every two days and water was added twice a week to replace that lost by evapotranspiration.

![Experimental design layout of the greenhouse container experiment using a completely randomized design (CRD) with four treatments, control (C), saline (S) and heat conditions (H) and the combination of salinity and heat (S + H), and six replicates per treatment.](image)

Figure 1. Experimental design layout of the greenhouse container experiment using a completely randomized design (CRD) with four treatments, control (C), saline (S) and heat conditions (H) and the combination of salinity and heat (S + H), and six replicates per treatment.

Plants were allowed to grow until they produced the ninth cluster, at which point the experiment terminated. Each tomato fruit was individually weighted to determine total and commercial production and mean fruit weight. Fruits under 70 g and/or affected by BER or cracking were classified as non-commercial. In order to analyze tomato quality, fruits at the full-red stage of ripening from trusses two and three were sampled during the period between 157 and 164 DAT. Fruit firmness of tomatoes with intact skin was determined with a texturometer (TA XT plus Texture Analyzer, Stable Micro System, Godalming, UK). Color was determined using a Minolta colorimeter CR200 model (Minolta Company, Limited, Ramsey, NJ, USA), taking three measurements for each fruit along the equatorial axis. Tomatoes taken from the same plant were cut into small pieces and mixed, constituting a sample (six samples per treatment). Later, the fruits were homogenized, and half of the homogenate was centrifuged to determine total soluble solids (TSS), pH and total acidity. The other half was kept at −80 °C for subsequent analysis of sugars, organic acids, vitamin C, phenolic compounds and carotenoids. Each sample was analyzed in triplicate.

Primary metabolites (soluble sugars and organic acids) and bioactive compounds (vitamin C, carotenoids and phenolic compound) were analyzed by high-performance liquid chromatography (HPLC) using a refraction index (IR) for sugars, a triple quadrupole mass spectrometer detector (MS/MS) for organic acids, vitamin C and phenolic compounds and a photodiode array UV-visible detector for carotenoids, following the methodologies described by Flores et al. [24], Fenoll et al. [25] and Flores et al. [26]. The IBM SPSS Statistic 21 software was used to statistically analyze the results with a one-way ANOVA and Duncan’s test.
3. Results and Discussion

3.1. Yield Parameters

The total tomato yield obtained under control conditions was significantly reduced by the three different treatments \((p < 0.001)\). The effect of salinity and heat individually was similar, and the combination of both stresses resulted in the highest yield reduction (Figure 2A). The reduction in commercial yield was even higher with all different treatments, which indicates a reduction in the percentage of commercial fruits (Figure 2B). Commercial yield was reduced from 91.8% under control conditions to 80.5, 73.5 and 65.4% with salinity, heat stress and the combination of both stresses, respectively. The above decrease in tomato yield with the different treatments was attributed to the significant reduction \((p < 0.001)\) in fruit weight (Figure 2C) and not to a reduction in fruit number (Figure 2D). Several authors have described a reduction in tomato fruit size but no or little effect on fruit number under saline conditions \([27–30]\). In regard to the decrease in fruit weight under saline conditions, this effect has been attributed to a lower water uptake by the root, thus reducing water transport to the fruit \([31–33]\). Unlike salinity, heat stress may affect fruit set with negative consequences for the yield \([34]\). However, under our experimental conditions, heat stress alone or combined with salinity had no significant effect on fruit number.

Figure 2. Total production (A), commercial production (B), fruit mean weight (C) and fruit number per plant (D) of tomato plants under control (C), saline (S) and heat conditions (H) or the combination of salinity and heat (S + H). Values are means ± SE \((n = 6)\). Different letters indicate significant differences between means according to Duncan’s test at the 5% level.

Different responses to combinations of stresses can be found: (1) additive, which is the addition of the single stress responses; (2) synergistic, which is the sum of each single stress; (3) idiosyncratic, when completely different from the individual stress responses; (4) dominant, if it is very close to one of the stresses \([35]\). Our results point to a higher negative effect of stress combinations than of each single stress on fruit yield (additive). Although Rivero et al. \([9]\) reported that after 72 h, the heat treatment improved the salinity tolerance of tomato plants, long-term exposure to stress, such as in the present study, would be expected to have more pronounced effects on plant physiology and fruit yield. In agreement with our results, other authors studying drought, heat and their combination in tomato plants over a period of 6 days indicated that combined stress reinforced the negative effect of the individual stresses \([36]\). In addition, long-term studies have indicated that different stress interactions have a higher effect on yield than any of the stresses applied individually \([8,11]\).
3.2. Fruit Organoleptic Properties

Total soluble solids (TSS) significantly increased by salinity, whether applied alone or, to a lesser extent, by the combination of salinity with high temperature, while temperature alone had no effect (Table 1). The combination of both stresses significantly decreased the pH in fruit, and the saline treatment applied as a single stress increased acidity. Other authors have reported similar results in relation to the effect of salt stress in tomato fruits, with both soluble solids and titratable acidity increasing [37,38]. Fruit firmness decreased with the combination of salinity and heat, but there were no differences between the other treatments. None of the treatments had any effect on L or hue values, while chroma increased only with the combination of stresses.

Table 1. Total soluble solids (TSS, °Brix), pH, acidity (g citric acid L⁻¹), firmness (N cm⁻²) and color parameters (L, hue and chroma) of tomato fruits under control (C), saline (S) and heat conditions (H) or the combination of salinity and heat (S + H). Values are means (n = 6).

| Treatments | TSS | pH   | Acidity | Firmness | L   | Hue | Chroma |
|------------|-----|------|---------|----------|-----|-----|--------|
| Control    | 4.7 | 4.3  | 2.0     | 13.8     | 42.9| 51.6| 39.5   |
| S          | 5.5 | 4.2  | 2.7     | 11.6     | 43.9| 54.1| 40.7   |
| H          | 4.6 | 4.4  | 2.0     | 13.5     | 43.3| 52.6| 39.7   |
| S + H      | 5.2 | 4.1  | 2.3     | 10.8     | 44.0| 51.2| 42.0   |

*,** Significant differences between means at the 5 or 1% level of probability, respectively; n.s., non-significant at p = 5%. For each stage, different letters in the same column indicate significant differences between means according to Duncan’s test at the 5% level.

The glucose and fructose contents significantly increased when salinity was applied as a single stress, but were not affected when heat was the only stress (Table 2). However, heat and salinity together had an additive effect, with the combination of both stresses resulting in the highest increase in both glucose and fructose. Many results can be found in the literature related to the increase in tomato fruit quality as a result of an increasing sugar content caused by salinity of the nutrient solution [3,27,39–41], which was attributed to the effect of saline stress on enzymes associated with sugar biosynthesis [42]. As for high temperature, no effect on the fruit’s reducing sugar content has been described in tomato in spite of its impact on fruit mass production [43]. However, our findings indicated that under high temperature conditions, irrigation with saline water could increase the fruit sugar content and, therefore, lead to greater consumer preference because of the increase in sweetness and flavor.

Table 2. Concentration of soluble sugars and organic acids (mg g⁻¹ fresh weight) in tomato fruits under control (C), saline (S) and heat conditions (H) or the combination of salinity and heat (S + H). Values are means (n = 6).

| Treatments | Glucose | Fructose | Citric | Glutamic | Malic |
|------------|---------|----------|--------|----------|-------|
| Control    | 14.75   | 15.40    | 1.59   | 3.00     | 0.42  |
| S          | 20.23   | 19.80    | 1.86   | 3.41     | 0.30  |
| H          | 13.64   | 14.32    | 1.95   | 2.23     | 0.49  |
| S + H      | 24.26   | 23.09    | 1.74   | 2.89     | 0.41  |

*,**,** Significant differences between means at 5 or 0.1% level of probability, respectively; n.s., non-significant at p = 5%. For each stage, different letters in the same column indicate significant differences between means according to Duncan’s test at the 5% level.

Glutamic acid concentration was not affected by any treatment with regard to the control (Table 2), although significant differences were found between single stress applications (p < 0.05), being 1.5 times higher under saline than under heat stress. In the case of malic acid, the heat treatment led to a 1.6 times higher content than that obtained in
saline conditions. Neither a single stress nor the combination of both significantly changed the citric acid concentration. Other authors have indicated that salinity increases organic acid as well as the sugar concentration [27,44], but this effect was closely dependent on the tomato variety [27]. The increased concentrations of both sugars and organic acids in tomato fruits by salinity and high temperature have been previously associated to a concentration effect as a result of a decreased sink/source ratio due to increased flower abortion [27,28]. However, the experimental conditions in the present study did not led to a decrease in the number of fruits as a consequence of any single stress or their combination. Therefore, the increased concentrations of sugars and organic acids could be attributed to an enhanced biosynthesis under these stress conditions.

3.3. Phenolic Compounds

The most abundant phenolic compound was homovanillic acid-O-hexoside, with an average concentration of 26.4 µg g⁻¹, followed by the flavonol derivative rutin (10.6 µg g⁻¹) and kaempferol-3-O-rutinoside (8.6 µg g⁻¹) and the flavanone naringenin (7.0 µg g⁻¹). Hydroxycinnamic acids were mainly represented by chlorogenic acid (5.9 µg g⁻¹). The dihydrochalcone phloretin-C-diglycoside was found at an average concentration of 3.8 µg g⁻¹. Other detected phenolic compounds were the flavonol derivatives rutin-O-hexoside (0.20 µg g⁻¹), rutin-O-pentoside (0.07 µg g⁻¹), quercetin (0.04 µg g⁻¹), the flavanone naringenin-O-hexoside (3.1 µg g⁻¹) and the hydroxycinnamic derivatives caffeic-acid-O-hexoside (2.4 µg g⁻¹), cryptochlorogenic acid (1.4 µg g⁻¹), ferulic acid-O-hexoside (1.3 µg g⁻¹) and p-coumaroyl quinic acid (0.19 µg g⁻¹), dicaffeoylquinic (0.15 µg g⁻¹), ferulic (0.14 µg g⁻¹), caffeic (0.12 µg g⁻¹) and p-coumaric acids (0.03 µg g⁻¹). Table S1 shows the values of each individual phenolic compound in the different treatments.

The content of hydroxycinnamic acids was significantly reduced by the high temperature, while the other treatments had no effect on these compounds (Figure 3A). Interestingly, salinity inhibited the detrimental effect of heat on this parameter. Flavanones were not affected by salinity but decreased significantly and in a similar manner with high temperature and the combination of salinity and heat (Figure 3B), which indicates that heat dominated the combined stress response. In contrast, flavonols were significantly increased by salinity and the combination of both stresses, and no significant differences were found between both conditions, indicating the dominant effect of salinity on this parameter. No effects of heat as a single stress were observed on the flavonol content (Figure 3C). Homovanillic acid-O-hexoside was significantly higher with both stresses applied together (Figure 4A), and phloretin was significantly reduced with the saline treatment (Figure 4B).

Phenolic compounds are important for the detoxification of free radicals [45] and environmental stress can increase the levels of these scavenger molecules [21]. Regarding salinity, contradictory reports of the effects on phenolic compounds in tomato fruits can be found in the literature, increasing [46,47], decreasing [48] or even remaining unchanged [49]. The same is true of flavonoids, with some authors finding an increase in the total flavonoid content of tomato fruits under saline conditions [48] and others reporting a reduction [50]. In the case of heat stress, some authors have pointed to an increase in specific phenolic compounds [6,51] under high temperature conditions. Martinez et al. [12] described a differential accumulation of phenolic compounds that was dependent on the type of abiotic stress, concluding that the accumulation of flavonols over hydroxycinnamic acids favored oxidative damage protection under abiotic stress. In agreement with these authors, our results indicated an increase in flavonols/hydroxycinnamic acids ratio under all stress conditions, with the highest values obtained when both stresses were combined.

The different results found in the literature could be due to the influence of several factors, such as stage of ripeness and tissue, growth conditions, genotype or the detection method [52,53]. Our results showed the specific effects of individual and combined stresses on each phenolic compound family, which may be underestimated when the total contents are analyzed with non-selective methods. Moreover, the effect of salinity on phenolic compounds may be influenced by other factors, as mentioned by Incerti et al. [54], who re-
ported that their level decreased or increased, depending on the season (spring or autumn). These findings highlight the need to study the interaction between different factors that are expected to coexist when evaluating the impact of abiotic stress on fruit composition.

**Figure 3.** Concentration of hydroxycinnamic acids (A) flavonoids (B) and flavonols (C) (µg g⁻¹ fresh weight) in tomato fruits under control (C), saline (S) and heat conditions (H) or the combination of salinity and heat (S + H). Values are means ± SE (n = 6). Different letters indicate significant differences between means according to Duncan’s test at the 5% level.

**Figure 4.** Concentration of homovanillic acid-O-hexoside (A) and phloretin (B) (µg g⁻¹ fresh weight) in tomato fruits under control (C), saline (S) and heat conditions (H) or the combination of salinity and heat (S + H). Values are means ± SE (n = 6). Different letters indicate significant differences between means according to Duncan’s test at the 5% level.
3.4. Vitamin C

Vitamin C concentrations increased \( (p < 0.01) \) similarly with high temperature and when both stresses were applied, indicating the dominant effect of heat stress and no effect of salinity (Figure 5). Gautier et al. [6] found that ascorbate levels decreased when temperature increased to 32 °C, and Rosales et al. [50] observed an increase in ascorbic acid under stress conditions due to high temperature. After an initial fall, an increase in vitamin C was found by Hernández et al. [55] after a long exposure to high temperatures, suggesting that plant metabolism adapted to a high temperature and/or when the temperature decreased during the night, a restoration of the ascorbate synthesis took place. Ehret et al. [30] suggested that vitamin C concentration increased as a response to abiotic stress through de novo synthesis or due to its regeneration from dihydrolipoic acid. In spite of the increase in vitamin C caused by salinity in tomato fruits reported by other authors [27,30,46,56], our results found that salinity had no effect when applied alone and no synergistic effect when applied at the same time as a high temperature.

![Figure 5. Concentration of vitamin C (µg g⁻¹ fresh weight) in tomato fruits under control (C), saline (S) and heat conditions (H) or the combination of salinity and heat (S + H). Values are means ± SE \((n = 6)\). Different letters indicate significant differences between means according to Duncan’s test at the 5% level.](image)

3.5. Carotenoids

Salinity applied as a single stress did not significantly affect any of the precursors or carotenoids (Figures 6 and 7). High temperature did not increase carotenoids concentrations while the concentration of phytoene (Figure 6A), phytofluene (Figure 6B) and violaxanthin (Figure 7D) decreased with heat, whether applied as a single stress or combined with salinity. In spite of what occurred with the individual stresses, lycopene and lutein increased as a response to the combination of both stresses (Figure 7A,C). As for \( \beta \)-carotene, no significant differences were observed between any of the single stress treatments or their combination and the control treatment (Figure 7B).

Carotenoids can contribute to the fluidity and permeability of membranes in response to changes in temperature [57,58]. Although heat stress (32 °C) caused a decrease in lycopene levels, under certain conditions the fruits could recover or even increase the initial concentrations [55]. High temperature seems to have no effect on \( \beta \)-carotene and lutein [6,55]. However, some authors have reported a beneficial effect of salinity on the carotenoid content, [27,30,40,59], while Serio et al. [60] reported that salinity did not affect the lycopene content, in agreement with our results. Comparative studies have indicated that the response of carotenoids in tomato to salinity was genotype dependent [50,59].

Unlike the response to salinity or high temperature when applied separately, a specific and different response to the combination of both stresses was the increase in lycopene and lutein concentrations. Under such stress conditions, our results suggested a degradation of the precursors phytoene and phytofluene towards the accumulation of lycopene and lutein and the maintenance of \( \beta \)-carotene levels at the expense of a decreased accumulation of violaxanthin. These results of increased lycopene and lutein concentrations are especially
important, considering the role of these metabolites in human health [61] and with lycopene being the principal carotenoid, which confers the red pigmentation to the fruit.

Figure 6. Concentration of phytoene (A) and phytofluene (B) (µg g\(^{-1}\) fresh weight) in tomato fruits under control (C), saline (S) and heat conditions (H) or the combination of salinity and heat (S + H). Values are means ± SE (n = 6). Different letters indicate significant differences between means according to Duncan’s test at the 5% level.

Figure 7. Concentration of lycopene (A), β-carotene (B), lutein (C) and violaxanthin (D) (µg g\(^{-1}\) fresh weight) in tomato fruits under control (C), saline (S) and heat conditions (H) or the combination of salinity and heat (S + H). Values are means ± SE (n = 6). Different letters indicate significant differences between means according to Duncan’s test at the 5% level.

The effect of a combination of stresses may differ from those of single stresses, highlighting the importance of studying the effect of stress interactions on the yield and quality of crops. To summarize our findings, salinity applied as a single stress decreased the yield of tomato but had a positive effect on fruit quality by increasing sugars and flavonols. High temperatures increased the vitamin C content, but had a negative effect on yield and the
content of various phenolic compounds (hydroxycinnamic acids and flavanones) and some carotenoids. Interestingly, an idiosyncrasy was found in the effect of the combination of stresses on the contents of homovallinic acid O-hexoside, lycopene and lutein. In addition, the combination of stresses inhibited the detrimental effect of high temperature on hydroxycinnamic acid content. The results from this preliminary study point to the viability of exploiting abiotic stresses and their combination to obtain tomatoes with increased levels of health-promoting compounds. However, it is to be expected that environmental, crop management and even varietal factors may affect the results obtained. Therefore, further studies are needed considering these factors and other abiotic stresses. Moreover, since abiotic stress combinations due to climate change are expected to severely restrict crop yield and fruit quality in the coming years, more studies that combine good crop management with new breeding tools and gene editing technologies will be needed in order to improve plant resilience and cope with the food, fiber and livestock feed demand.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture11060534/s1, Table S1: Concentration of individual phenolic compounds (µg g⁻¹ fresh weight) in tomato fruits under control, salinity, heat or the combination of salinity and heat. Values are means ± SE (n = 6). Different letters indicate significant differences between means according to Duncan’s test at the 5% level.

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References
1. Botella, M.Á.; Del Amor, F.; Amorós, A.; Serrano, M.; Martinez, V.; Cedrá, A. Polyamine, ethylene, and other physico-chemical parameters in tomato (Lycopersicon esculentum) fruits as affected by salinity. Physiol. Plant. 2000, 109, 428–434. [CrossRef]
2. Petersen, K.K.; Willumsen, J.; Kaack, K. Composition and taste of tomatoes as affected by increased salinity and different salinity sources. J. Hortic. Sci. Biotechnol. 1998, 73, 205–215. [CrossRef]
3. Restuccia, G.; Marchese, M.; Mauromicali, G.; Restuccia, A.; Battaglia, M. Yield and Fruit Quality of Tomato Grown in Greenhouse with Saline Irrigation Water. Acta Hortic. 2003, 614, 699–704. [CrossRef]
4. Adams, S.R.; Cockshull, K.E.; Cave, C.R.J. Effect of Temperature on the Growth and Development of Tomato Fruits. Ann. Bot. 2001, 88, 869–877. [CrossRef]
5. Islam, M.T. Effect of Temperature on Photosynthesis, Yield Attributes and Yield of Tomato Genotypes. J. Exp. Agric. Int. 2011, 2, 8–11.
6. Gautier, H.; Diakou-Verdin, V.; Bénard, C.; Reich, M.; Buret, M.; Bourgaud, F.; Poéssel, J.L.; Caris-Veyrat, C.; Génard, M. How Does Tomato Quality (Sugar, Acid, and Nutritional Quality) Vary with Ripening Stage, Temperature, and Irradiance? J. Agric. Food Chem. 2008, 56, 1241–1250. [CrossRef]
7. Arena, C.; Conti, S.; Francesca, S.; Melchiomena, G.; Hájek, J.; Barták, M.; Barone, A.; Rigano, M.M. Eco-Physiological Screening of Different Tomato Genotypes in Response to High Temperatures: A Combined Field-to-Laboratory Approach. Plants 2020, 9, 508. [CrossRef]
Agriculture 2021, 11, 534

35. Prasch, C.M.; Sonnewald, U. Signaling events in plants: Stress factors in combination change the picture. *Environ. Exp. Bot.* 2015, 114, 4–14. [CrossRef]

36. Zhou, R.; Kong, L.; Wu, Z.; Rosenqvist, E.; Wang, Y.; Zhao, L.; Zhao, T.; Ottosen, C.-O. Physiological response of tomatoes at drought, heat and their combination followed by recovery. *Physiol. Plant.* 2018, 165, 144–154. [CrossRef] [PubMed]

37. Balibre, M.E.; Cuartero, J.; Bolarin, M.C.; Pérez-Alfocea, F. Sucrolytic activities during fruit development of Lycopersicon genotypes differing in tolerance to salinity. *Physiol. Plant.* 2003, 118, 38–46. [CrossRef] [PubMed]

38. Zushi, K.; Matsuzoe, N. Metabolic profile of organoleptic and health-promoting qualities in two tomato cultivars subjected to salt stress and their interactions using correlation network analysis. *Sci. Hortic.* 2015, 184, 8–17. [CrossRef]

39. Flores, P.; Navarro, J.M.; Carvajal, M.; Cerdà, A.; Martínez, V. Tomato yield and quality as affected by nitrogen source and salinity. *Agronomy* 2003, 23, 249–256. [CrossRef]

40. Fanasca, S.; Martino, A.; Heuvelink, E.; Stanghellini, C. Effect of electrical conductivity, fruit pruning, and truss position on quality in greenhouse tomato fruit. *J. Hortic. Sci. Biotechnol.* 2007, 82, 488–494. [CrossRef]

41. Zhang, P.; Senge, M.; Dai, Y. Effects of Salinity Stress on Growth, Yield, Fruit Quality and Water Use Efficiency of Tomato Under Hydroponics System. *Rev. Agric. Sci.* 2016, 4, 46–55. [CrossRef]

42. Saito, T.; Fukuda, N.; Matsukura, C.; Nishimura, S. Effects of Salinity on Distribution of Photosynthates and Carbohydrate Metabolism in Tomato Grown using Nutrient Film Technique. *J. Jpn. Soc. Hortic. Sci.* 2009, 78, 90–96. [CrossRef]

43. Kläring, H.-P.; Klopotek, Y.; Krumbein, A.; Schwarz, D. The effect of reducing the heating set point on the photosynthesis, growth, yield and fruit quality in greenhouse tomato production. *Agric. For. Meteorol.* 2015, 214–215, 178–188. [CrossRef]

44. Zhang, P.; Senge, M.; Yoshiyama, K.; Ito, K.; Dai, Y.; Zhang, F. Effects of Low Salinity Stress on Growth, Yield and Water Use Efficiency of Tomato Under Soilless Cultivation. *IDRE J.* 2017, 85, 15–21. [CrossRef]

45. Ksouri, R.; Megdiche, W.; Krumbain, A.; Schwarz, D. The effect of reducing the heating set point on the photosynthesis, growth, yield and fruit quality in greenhouse tomato production. *Agric. For. Meteorol.* 2015, 214–215, 178–188. [CrossRef]

46. Sgherri, C.; Navari-Izzo, F.; Pardossi, A.; Soressi, G.P.; Izzo, R. The Influence of Diluted Seawater and Ripening Stage on the Content of Antioxidants in Fruits of Different Tomato Genotypes. *J. Agric. Food Chem.* 2007, 55, 2452–2458. [CrossRef]

47. Krauss, S.; Schnitzler, W.H.; Grassmann, A.J.; Woitke, M. The Influence of Different Electrical Conductivity Values in a Simplified Recirculating Soilless System on Inner and Outer Fruit Quality Characteristics of Tomato. *J. Agric. Food Chem.* 2006, 54, 441–448. [CrossRef]

48. Hernández-Fuentes, A.D.; López-Vargas, E.R.; Pinedo-Espinosa, J.M.; Campos-Montiel, R.G.; Valdés-Reyna, J.; Juárez-Maldonado, A. Postharvest Behavior of Bioactive Compounds in Tomato Fruits Treated with Cu Nanoparticles and NaCl Stress. *Appl. Sci.* 2017, 7, 980. [CrossRef]

49. Kim, H.-J.; Fonseca, J.M.; Kubota, C.; Kroggel, M.; Choi, J.-H. Quality of fresh-cut tomatoes as affected by salt content in irrigation water and post-processing ultraviolet-C treatment. *J. Sci. Food Agric.* 2008, 88, 1969–1974. [CrossRef]

50. Moles, T.M.; Francisco, R.D.B.; Mariotti, L.; Pompeiano, A.; Lupini, A.; Incrocci, L.; Carmassi, G.; Scartazza, A.; Pistelli, L.; Guglielmetti, L.; et al. Salinity in Autumn-Winter Season and Fruit Quality of Tomato Landraces. *Front. Plant Sci.* 2019, 10, 1078. [CrossRef]

51. Rosales, M.A.; Cervilla, L.M.; Sánchez-Rodriguez, E.; Rubio-Wilhelmi, M.D.M.; Blasco, B.; Rios, J.J.; Soriano, T.; Castillo, N.; Romero, L.; Ruiz, J.M. The effect of environmental conditions on nutritional quality of cherry tomato fruits: Evaluation of two experimental Mediterranean greenhouse. *J. Sci. Food Agric.* 2010, 91, 152–162. [CrossRef] [PubMed]

52. Slimestad, R.; Verheul, M.J. Seasonal Variations in the Level of Plant Constituents in Greenhouse Production of Cherry Tomatoes. *J. Agric. Food Chem.* 2005, 53, 3114–3119. [CrossRef] [PubMed]

53. Slimestad, R.; Fossen, T.; Verheul, M.J. The Flavanoids of Tomatoes. *J. Agric. Food Chem.* 2008, 56, 2436–2441. [CrossRef]

54. Incerti, A.; Navari-Izzo, F.; Pardossi, A.; Izzo, R. Seasonal variations in polyphenols and lipic acid in fruits of tomato irrigated with seawater. *J. Sci. Food Agric.* 2009, 89, 1326–1331. [CrossRef]

55. Hernández, V.; Hellin, P.; Fenoll, J.; Flores, P. Increased Temperature Produces Changes in the Bioactive Composition of Tomato, Depending on Its Developmental Stage. *J. Agric. Food Chem.* 2015, 63, 2378–2382. [CrossRef]

56. Zushi, K.; Matsuzoe, N. Effect of Soil Water Deficit on Vitamin C, Sugar, Organic Acid, Amino Acid and Carotene Contents of Large-fruited Tomatoes. *J. Jpn. Soc. Hortic. Sci.* 1998, 67, 927–933. [CrossRef]

57. Nisar, N.; Li, L.; Lu, S.; Khin, N.C.; Pogson, B.J. Carotenoid Metabolism in Plants. *Mol. Plant.* 2015, 8, 68–82. [CrossRef] [PubMed]

58. Spicher, L.; Glauser, G.; Kessler, F. Lipid Antioxidant and Galactolipid Remodeling under Temperature Stress in Tomato Plants. *Front. Plant Sci.* 2016, 7, 167. [CrossRef] [PubMed]

59. Borghesi, E.; González-Miret, M.L.; Escudero-Gilete, M.L.; Malorgio, F.; Heredia, F.J.; Melendez-Martinez, A.J. Effects of Salinity Stress on Carotenoids, Anthocyanins, and Color of Diverse Tomato Genotypes. *J. Agric. Food Chem.* 2011, 59, 11676–11682. [CrossRef] [PubMed]

60. Serio, F.; De Gara, L.; Caretto, S.; Leo, L.; Santamaria, P. Influence of an increased NaCl concentration on yield and quality of cherry tomato grown in Posidonia (*Posidonia oceanica (L.) Delile*). *J. Sci. Food Agric.* 2004, 84, 1885–1890. [CrossRef]

61. Przybylska, S. Lycopene—A bioactive carotenoid offering multiple health benefits: A review. *Int. J. Food Sci. Technol.* 2020, 55, 11–32. [CrossRef]