



# Nutrient Composition, Antioxidant Components and Ascorbic Acid Content Response of Pepper Fruit (*Capsicum annuum* L.) Cultivars Grown under Salt Stress

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## Abstract

Salinity stress occurs due to the accumulation of high levels of salts in soil, which ultimately leads to the impairment of plant growth and crop loss. Stress tolerance-inducing compounds have a remarkable ability to improve growth and minimize the effects of salinity stress without negatively affecting the environment by controlling the activities in plants. The pots were arranged in a complete randomized design with one plant per pot and four replicates per treatment and carried out in 2017 and 2018 to study the influence of four levels of NaCl (0, 50, 100 and 200 mM) on the antioxidant, ascorbic acid, organic and inorganic compounds of three pepper fruits cultivars (“Granada”, “Goliath” and “Nobili”) at mature stage. The results obtained showed that salinity decreased the mineral content, relative water content, and agro-morphological parameters of pepper fruit. This decrease was accompanied by a significant increase of Na, soluble proteins, proline content, fructose, glucose and antioxidants, including total phenolics and flavonoids, and reduced ascorbic acid and  $\beta$ -carotene content. However, a varietal difference response to salt stress was observed between the studied varieties. Indeed, the variety Granada is characterized by their vigour in absence as in the presence of salt. Under the studied salinity level there was an enhancement of health-promoting compounds (phenolic compounds, flavonoids, and soluble sugar) synthesis in pepper fruits, with significant changes in other quality parameters. “Granada” was more tolerant and stable in physiological, biochem-

ical and agro-morphological traits suggesting that it could be grown in salt-affected soils.

### Subject Areas

Agricultural Science

### Keywords

Agro-Morphological Parameters, Antioxidant, Ions Distribution, Organic Compound, Salinity

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## 1. Introduction

Salinity is one of the most important environmental factors that affect the distribution and abundance of plant species. Soil salinization occurs mainly in two ways: high evaporation relative to precipitation in association with weak leaching in soils, and salt accumulation as a result of the use of saline water [1]. It is estimated that about 50% of the world's land will be saline by the middle of the 21<sup>st</sup> century [2]. Globally, 20% of irrigated land and 2.1% of dry land agriculture suffers from the salt problem and NaCl is the predominant salt causing salinization [3]. Salinity adversely affects germination, growth, physiology and productivity by reducing the ability of plants to take up water causing foliage damage and even death of the plants, imbalance in osmotic potential; ionic equilibrium and nutrient uptake [4]. Further, it facilitates severe ion toxicity by depositing high concentration of Na<sup>+</sup> which causes membrane disorganization, inhibition of cell division and expansion. The influence of salinity and mineral nutrient solution, on productivity, photosynthesis and growth has been studied in different plants [5] [6]. It stated that high levels of Na<sup>+</sup> inhibit K, Ca and Mg in leaves, which results in a K/Na antagonism and net photosynthesis is affected strongly by NaCl conditions, which is related directly to the closure of stomata as to low intercellular CO<sub>2</sub> levels [7]. To develop saline zones and/or the zones having only brackish water resources, it is important to select tolerant varieties. Salinity imposes stress conditions on crop plants, affects growth and chemical contents and has been shown to limit pepper yield [8]. Salt stress severely inhibits plant growth for two reasons: firstly due to an osmotic or water-deficit effect of salinity and secondly due to a salt-specific or ion excess effect of NaCl. Soils with high levels of salinity have a low water potential zone; consequently, it is difficult for the plant to absorb water and nutrients. In other words saline soils expose plants to osmotic stress [9]. One of the most important consequences of osmotic stress on plants is the production of reactive oxygen species (ROS) in large amounts that followed by oxidative damages, the degradation of proteins, lipids, pigments, and DNA [10]. Plants growing in saline conditions take up harmful ions, especially Na<sup>+</sup> and Cl<sup>-</sup> ions. Accumulation of Na<sup>+</sup> and Cl<sup>-</sup> ions in large amounts is toxic for the cell, and compounds osmotic stress [9]. These ions disrupt mem-

brane integrity, cell metabolism, enzyme structure, cell growth, and photosynthesis [11]. Although plants have a variety of ways of withstanding the stress, significant loss of yield occurs [12]. Salt stress is known to negatively affect plant growth at all developmental stages, but sensitivity varies greatly at different stages [13]. Crop production in saline areas largely depends on successful germination, seedling emergence and establishment and efficient reproductive phase [13]. Moreover, as environmental stress it may have a strong influence on the concentration of bioactive compounds of vegetables [14]. Meanwhile, there is an increasing need to produce enough food for the world's growing population [1]. In order to address these challenges to the world's food security, the engineering of plants to create species that tolerate salinity has been considered as a promising strategy.

Pepper (*Capsicum annuum* L.) is an important agricultural crop, not only because of its economic importance, but also for the nutritional value of its fruits; it is an excellent source of natural colors, vitamin C and antioxidant compounds important for human health [15]. Pepper is a moderately sensitive to salt stress [16], and it is grown under protected glasshouse conditions in temperate regions and in the open field under warm Mediterranean climates. Salinity imposes stress conditions on crop plants [17] and affects growth and chemical contents and has been shown to limit pepper yield [8]. Salt stress severely inhibits plant growth for two reasons: first by an osmotic or water-deficit effect of salinity and second by a salt-specific or ion excess effect of NaCl. Moreover, plants subject to salinity stress conditions produce cytotoxic activated oxygen that can seriously disrupt normal metabolism, through oxidative damage of lipids, proteins, and nucleic acids [18]. To defend against such oxidants, plants have evolved specific protective mechanisms, involving antioxidant molecules and enzymes that protect against the potentially-cytotoxic species of activated oxygen. In case of high salinity, oxidative stress occurs due to closure of stomata, interruption of photosynthetic electron transport and disruption of cellular membrane integrity and antioxidative defense systems of plants start work against oxidative damage. The antioxidants include non-enzymatic ones, such as vitamin E, vitamin C, glutathione and carotenoid ( $\beta$ -carotene and zeaxanthin), and enzymes such as superoxide dismutase and catalase [19].

The objective of this study was to investigate the effect of NaCl treatment on nutrient composition, antioxidant components, ascorbic acid content, and agro-morphological parameters of three varieties of pepper fruit (*Capsicum annuum* L.) in order to better understand their differences on salt stress tolerance and select tolerant varieties which could be cultivated in arid, semi-arid and coastal saline soils.

## 2. Materials and Methods

### 2.1. Plant Materials

Pepper (*Capsicum annuum* L.) is especially productive in warm and dry climates

than *C. frutescens* which can tolerate most climates. It also displays a greater resistance to disease and insects, especially to the tobacco mosaic virus. Capsaicinoids chemicals and antioxidants such as carotenoids provide the distinctive tastes in *C. annuum* variants. The fruit are berries that may be green, yellow or red when ripe. The mature green stage is ideal to acquire maximum pungency due to capsaicinoids, whereas peppers at red ripe stage are best sources of ascorbic acid and dried fruits contain higher levels of total carotenoids [20]. Hot peppers are used in medicine as well as food in Africa. Seeds of three pepper cultivars (“Granada”, “Goliath” and “Nobili”), provided by the breeding program of the Agronomic Institute for Research and Development of Cameroon were used in the study. These varieties were chosen for their socio-economic rank and resistance to the tobacco mosaic virus.

## 2.2. Plant Growth Conditions and Salt Treatments

The present work was performed in the greenhouse of the Faculty of Science at University of Douala, Cameroon, from September 2017 to August 2018. The seeds were surface sterilized with 3% sodium hypochlorite for 20 min and washed four times with deionized water. One-month-old pepper seedlings were transplanted into 10-L plastic pots filled with 5 kg of sterilized sand. The pots were arranged in a complete randomized design with one plant per pot and four replicates per treatment. All plants were fertilized daily with a modified nutrient solution (in  $\text{g}\cdot\text{L}^{-1}$ ): 150 g  $\text{Ca}(\text{NO}_3)_2$ , 70 g  $\text{KNO}_3$ , 15 g Fe-EDTA, 0.14 g  $\text{KH}_2\text{PO}_4$ , 1.60 g  $\text{K}_2\text{SO}_4$ , 11 g  $\text{MgSO}_4$ , 2.5 g  $\text{CaSO}_4$ , 1.18 g  $\text{MnSO}_4$ , 0.16 g  $\text{ZnSO}_4$ , 3.10 g  $\text{H}_3\text{BO}_3$ , 0.17 g  $\text{CuSO}_4$  and 0.08 g  $\text{MoO}_3$  [21]. The pH of the nutrient solution was adjusted to 7.0 by adding  $\text{HNO}_3$  0.1 mM. For the determination of physiological and biochemical responses of pepper cultivars to salt stress, each cultivar was subjected to 0 (control), 50, 100 and 200 mM NaCl. Plants were watered with deionized water every morning. The daily amounts of water added to the pots were the same for all treatments. Throughout the growth period, average day/night temperatures in the greenhouse were 26°C/20°C and the relative air humidity averaged 68.5%.

## 2.3. Plant Measurements

Plant samples were harvested after 4 months culture under salt stress, fruits were collected. The tissues (fruits) were dried for 24 h at 105°C [22]. The dry samples were weighted. Ninety days after sowing, samples from each treatment were collected to determine agro-morphological characters (number of fruit per plant, fresh fruit weight of fruit, dry fruit weight, thickness of fruit, fruit length, fruit diameter), organic components (total soluble proteins, proline, fructose, glucose), inorganic components (Na, K, Ca, Mg, P, S, Zn, Cu, Mn, Fe content, K/Na, Ca/Na, Mg/Na), relative water content, antioxidant levels (total flavonoid content, total phenolic content, ascorbic acid,  $\beta$ -carotene content) in three pepper fruit cultivars.

### **Agromorphological characters**

90 days after transplanting, the fresh fruit weight (FFW) and dry fruit weight (DFW) were determined. The number of fruit per plant, the fruit length and diameter were measured [23].

### **Total soluble proteins**

Protein content was determined by Bradford's method [24]. Briefly, appropriate volume (from 0 - 100  $\mu$ l) of sample was aliquoted into a tube and the total volume was adjusted to 100  $\mu$ l with distilled water. A 1 ml of Bradford working solution was added to each sample well. Then the mixture was thoroughly mixed by vortex mixer. After left for 2 min, the absorbance was read at 595 nm. The standard curve was established by replacing the sample portions in the tubes with proper serial dilutions of bovine serum albumin.

### **Free proline**

Free proline was determined in 95% ethanol extracts from fruits. Samples of 0.5 g of tissues freshly harvested were crushed in 5 ml 95% (v/v) ethanol. The insoluble fraction of the extract was washed twice with 5 ml of 70% ethanol. All soluble fractions were centrifuged at 3500  $\times$  g for 10 min. The supernatants were collected and stored at 4°C for proline determination [25]. The free proline content was measured [26].

### **Total soluble sugar**

Glucose and fructose were extracted from pepper fruit homogenate aliquots according to the protocol described in [27], and then quantified enzymatically [28].

### **Minerals**

P, K, Ca, Mg, S and Na contents in the fruit tissue of the plants were evaluated in dry, ground, and digested samples in a CEM microwave oven [29]. P was determined by colorimetry; sodium and potassium by flame photometry; copper determination was carried out by means of mass spectrometry with source of plasma connected by induction (ICP-MS), by means of a Hewlett Packard 4500 series; calcium and magnesium by atomic absorption spectrometry and sulfur by turbidimetry of barium sulfate [30]. Iron, zinc and manganese contents were determined by method reported in [31]. Fruit of pepper was dry ashed at 450°C for 2 hours and digested on heat cave with 10 ml HNO<sub>3</sub> 1 M. The solution was filtrated and adjusted at 100 ml with HNO<sub>3</sub> at 1/100 and analyzed with an atomic absorption spectrophotometer (Rayleigh, WFX-100).

### **Relative water content**

The relative water content (RWC) in fruits was recorded according to the formula as follows:  $RWC = (FFW - FDW)/(TW - FDW) \times 100$ , where FFW is fresh weight, FDW is dry weight, and TW is turgid weight [32].

### **Total flavonoid content**

FLA content of crude extract was determined by the aluminium chloride colorimetric method [33]. 50  $\mu$ L of crude extract (1 mg/mL ethanol) were made up to 1 mL with methanol, mixed with 4 mL of distilled water and then 0.3 mL of

5% NaNO<sub>2</sub> solution; 0.3 mL of 10% AlCl<sub>3</sub> solution was added after 5 min of incubation, and the mixture was allowed to stand for 6 min. Then, 2 mL of 1 mol/L NaOH solution were added, and the final volume of the mixture was brought to 10 mL with double-distilled water. The mixture was allowed to stand for 15 min, and absorbance was recorded on spectrophotometer (Pharmaspec UV-1700 model) at 510 nm wavelength. FLA content was calculated from a rutin calibration curve, and the result was expressed as g rutin equivalent per g dry weight.

#### **Total phenolic content**

TP content of the extract was determined by the Folin Ciocalteu method [34]. Subsamples (1 g) of fresh fruits were ground at 4°C in 3 mL of 0.1 N HCl. After incubation to 4°C for 20 min, the homogenate was centrifuged at 6000 g for 40 min. The supernatant was collected, the pellet re-suspended in 3 mL of 0.1 N HCl and centrifuged as previously. The two supernatant are mixed and constitute the crude extract of soluble phenol. The reaction mixture containing 15 µL of extract, 100 µL Folin-Ciocalteu reagents, 0.5 mL of 20% Na<sub>2</sub>CO<sub>3</sub>, was incubated at 40°C for 20 min and absorbance read at 720 nm wavelength with a spectrophotometer (Pharmaspec UV-1700 model). A standard curve was established using chlorogenic acid. TP content was expressed as mg g<sup>-1</sup> fresh weight.

#### **Ascorbic acid content**

For estimation of ascorbic acid content (ASA), 1 g of frozen fruit tissues was homogenised in 5 mL of ice-cold 6% m-phosphoric acid (pH 2.8) containing 1 mM EDTA [35]. The homogenate was centrifuged at 20,000 × g for 15 min at 4°C. The supernatant was filtered through a 30-µm syringe filter, and 50 µL of the filtrate was analyzed using an HPLC system (PerkinElmer series 200 LC and UV/VIS detector 200 LC, USA) equipped with a 5-µm column (Spheri-5 RP-18; 220 × 4.6 mm; Brownlee) and UV detection at 245 nm with 1.0 mL/min water (pH 2.2) as the mobile phase, run isocratically [36].

#### **β-carotene content**

β-carotene was extracted by grinding fruit tissues in a solution of 100% acetone containing CaCO<sub>3</sub> [37]. The extracts were centrifuged at 16,000 × g for 10 min, and 20 µL of the resulting supernatants were used for HPLC analysis, as described by [38] using the previously mentioned HPLC system. Solvent A (acetonitrile, methanol, Tris-HCl buffer 0.1 M, pH 8.0, 72:8:3) was run isocratically from 0 to 4 min followed by a 2.5 min linear gradient to 100% solvent B (methanol, hexane, 4:1) at a flow rate of 2 mL/min. The detector was set at 440 nm for the integration of peak areas after calibration with the external standard.

#### **Experimental design and statistical analysis**

The experiment was conducted as a factorial completely randomized design with four NaCl treatments and three cultivars in four replications. Data are presented in term of mean (±standard deviation). All data were statistically analysed using Statistica (version 9, Tulsa, OK, USA) and first subjected to analyses of variance (ANOVA). Statistical differences between treatment means were estab-

lished using the Fisher LSD test at  $p < 0.05$ .

### 3. Results and Discussion

#### 3.1. Agro-Morphological Parameters

According to findings in **Table 1**, salt stress at 100 and 200 mM NaCl caused significant decreases in NF, FFW, FDW, TF, FL and FD. The lowest values of these traits were recorded with salt stressed plants at 200 mM concentration, followed by 100 mM. In “Granada”, “Goliath” and “Nobili” there was a gradual decrease in the fruit length and diameter per plant following the increase in NaCl salinity. The cultivar “Granada” presented the higher FL 18.86 cm for control and 14.84 cm at 200 mM NaCl and “Goliath” present higher FD, 8.84 to 6.37 cm, when applying the highest of NaCl (200 mM) (**Table 1**). The NF decreased linearly with the increase in the salinity, with significant effect on both cultivars (**Table 1**). The NF of the cultivar “Goliath”, 11.41 to 6.89, were higher than those of the cultivar “Nobili”, 10.28 to 7.68 and “Granada”, 9.81 to 7.23, when applying the highest of NaCl (200 mM). The cultivar “Granada” showed higher FFW, varied within a range of 45.55 to 37.81 g respectively to control and 200 mM NaCl (**Table 1**). These results were directly related to FDW, which decreased with increasing salinity (**Table 1**). The TF losses of the cultivars reached 30.06% in

**Table 1.** Effect of salinity on some agro-morphological parameters of pepper fruit (Fruit Length, Fruit Diameter, Number of Fruit, Thickness of Fruit, Fresh Fruit Weight and Fruit Dry Weight) at the mature stage (90 DAP).

Cultivar	Treatment (mM NaCl)	Fruit Length (cm)	Fruit Diameter (cm)	Number of Fruit	Fresh Fruit Weight (g)	Dry Fruit Weight (g)	Thickness of Fruit (mm)
Granada	0	18.86 ± 0.14 <sup>a</sup>	5.98 ± 0.09 <sup>b</sup>	12.82 ± 0.18 <sup>a</sup>	45.55 ± 2.25 <sup>a</sup>	4.03 ± 0.07 <sup>a</sup>	5.82 ± 0.07 <sup>ab</sup>
	50	16.89 ± 0.18 <sup>ab</sup>	4.54 ± 0.11 <sup>b</sup>	11.22 ± 0.19 <sup>a</sup>	43.81 ± 2.30 <sup>a</sup>	3.15 ± 0.04 <sup>a</sup>	5.78 ± 0.08 <sup>ab</sup>
	100	14.12 ± 0.15 <sup>b</sup>	3.77 ± 0.08 <sup>bc</sup>	8.38 ± 0.15 <sup>b</sup>	39.74 ± 2.01 <sup>b</sup>	1.88 ± 0.03 <sup>b</sup>	4.32 ± 0.06 <sup>b</sup>
	200	14.84 ± 0.11 <sup>b</sup>	4.08 ± 0.12 <sup>b</sup>	7.93 ± 0.21 <sup>b</sup>	37.81 ± 1.88 <sup>b</sup>	1.61 ± 0.04 <sup>b</sup>	4.07 ± 0.07 <sup>b</sup>
Nobili	0	13.68 ± 0.09 <sup>b</sup>	7.16 ± 0.13 <sup>a</sup>	11.62 ± 0.22 <sup>a</sup>	37.67 ± 1.92 <sup>b</sup>	3.74 ± 0.05 <sup>a</sup>	7.27 ± 0.18 <sup>a</sup>
	50	12.16 ± 0.08 <sup>bc</sup>	6.87 ± 0.15 <sup>a</sup>	10.09 ± 0.23 <sup>a</sup>	32.32 ± 1.89 <sup>d</sup>	2.04 ± 0.07 <sup>ab</sup>	6.66 ± 0.16 <sup>a</sup>
	100	10.64 ± 0.07 <sup>d</sup>	4.83 ± 0.18 <sup>b</sup>	8.63 ± 0.21 <sup>b</sup>	35.22 ± 1.99 <sup>c</sup>	1.05 ± 0.08 <sup>b</sup>	5.15 ± 0.09 <sup>ab</sup>
	200	9.88 ± 0.10 <sup>d</sup>	4.29 ± 0.21 <sup>b</sup>	7.68 ± 0.24 <sup>b</sup>	27.43 ± 1.49 <sup>e</sup>	0.92 ± 0.04 <sup>b</sup>	4.91 ± 0.11 <sup>b</sup>
Goliath	0	10.77 ± 0.11 <sup>d</sup>	8.84 ± 0.07 <sup>a</sup>	11.41 ± 0.2 <sup>a</sup>	31.27 ± 1.87 <sup>d</sup>	2.38 ± 0.07 <sup>ab</sup>	7.78 ± 0.14 <sup>a</sup>
	50	8.93 ± 0.08 <sup>de</sup>	6.96 ± 0.09 <sup>a</sup>	8.23 ± 0.19 <sup>b</sup>	23.89 ± 1.66	0.81 ± 0.02 <sup>b</sup>	5.89 ± 0.06 <sup>ab</sup>
	100	6.77 ± 0.07 <sup>e</sup>	7.11 ± 0.12 <sup>a</sup>	7.81 ± 0.18 <sup>b</sup>	25.65 ± 1.27 <sup>e</sup>	0.93 ± 0.01 <sup>b</sup>	6.04 ± 0.09 <sup>a</sup>
	200	6.88 ± 0.06 <sup>e</sup>	6.37 ± 0.11 <sup>a</sup>	6.89 ± 0.20 <sup>c</sup>	20.33 ± 1.09 <sup>f</sup>	0.73 ± 0.02 <sup>c</sup>	5.60 ± 0.10 <sup>ab</sup>
Two way ANOVA results							
Cultivar (C)		*	*	NS	**	*	NS
Salt treatment (S)		**	*	*	*	*	*
Interaction C X S		NS	NS	NS	*	NS	NS

Values shown are means ( $n = 10$ ) ± SD; within columns, means followed by different letter are significantly different ( $p < 0.05$ ). \*\*, \* significant at 1% and 5% probability levels, respectively, NS not significant.

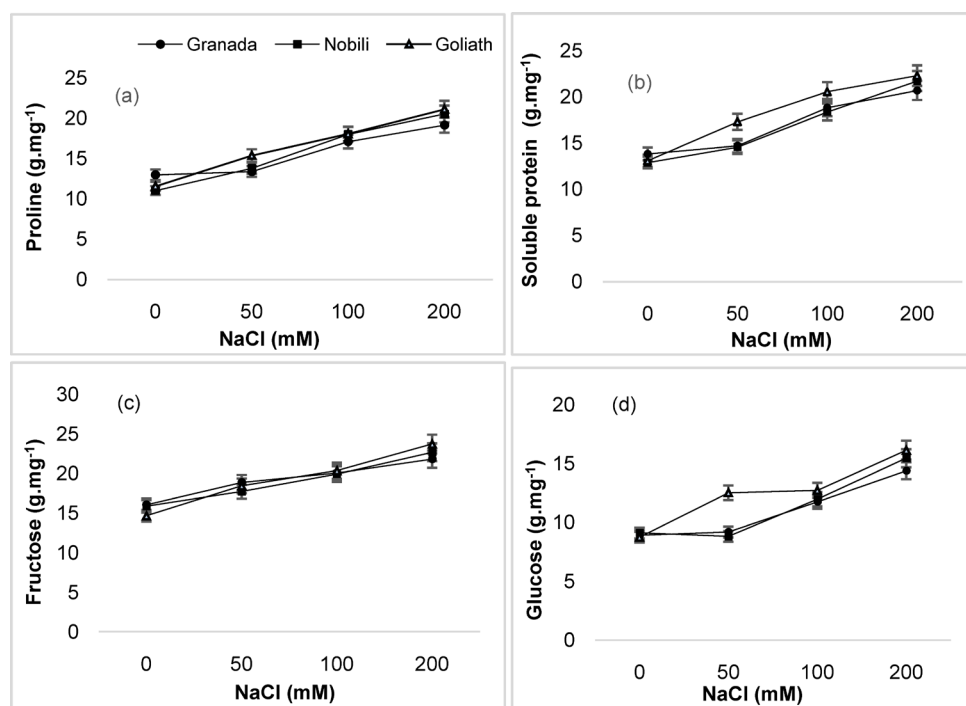


“Granada”, 32.46% in “Nobili” and 28.02% in “Goliath” under the highest NaCl (200 mM). Under salinity stress of 200 mM, the best results of NF, FFW, FDW, FL and FD were recorded with “Nobili”, followed by “Granada” and “Goliath”. Similar results were also reported for tomato [39] and strawberry [40] grown in saline soil. In contrast, several authors reported that FDW significantly increased under saline conditions in a number of horticultural crop species including tomato [41] and cucumber [42]. The reductions of NF, FFW, FDW, TF, FL, FD under salt conditions are possibly due to the adverse impacts of salinity on the growth characteristics and physiological processes such as water uptake, photosynthesis, flowering, and fruit formation, which led to diminished yields. Accordingly, the highest level of salt (200 mM NaCl) was adversely more effective than the lowest one (50 mM NaCl). The same trends of salt stress were previously described in faba bean [43] and strawberry plants [44]. Pulp thickness and firmness of pepper fruit are an important variable affecting pepper quality, since it guarantees better postharvest conservation and prevents injury by manipulation. High salinity, above 200 mM NaCl, tended to decrease pulp thickness and fruit firmness. Firmness in pepper fruit has been related to the level of calcium in the fruit [45]. Thus, salinity could reduce fruit pulp thickness and firmness by reducing the availability of calcium in the fruit.

### 3.2. Organic Compounds

The presence of NaCl resulted in a significant increase in GLU, FRU, SP and PRO contents in fruit of all cultivars compared to untreated plants, thereby playing a major role as osmotic adjustment (Figures 1(a)-(d)). The concentration of free sugars is one of the components along with organic acids that determine fruit flavor attributed to sweetness of pepper fruit. Both FRU and GLU composition differed by level of NaCl and varieties. The level of fructose across all samples was higher than that of GLU (Figure 1(c) and Figure 1(d)). The contents of FRU and GLU across varieties were higher in pepper produced at 0 mM NaCl in “Granada” and “Nobili”, at 200 mM “Granada” than those of “Goliath” and other level of NaCl. SP content in plants increased significantly under salt stress in all cultivars compared to untreated plants (Figure 1(b)). These increases were 49.71% in “Granada”, 68.55% in “Nobili” and 70.23% in “Goliath” at 200 mM NaCl in comparison with the control plants. The proline content had markedly accumulated in sweet pepper fruits; the highest concentration was recorded with a salinity at 200 mM NaCl (47.64% in “Granada”, 86.29% in “Nobili” and 100.18% in “Goliath” in comparison to the control plants (Figure 1(a)). The salt tolerant “Granada” accumulated the highest amount of all osmolytes followed by the moderately tolerant “Nobili” and the salt-sensitive “Goliath”. [46] observed an increase in protein content when increasing salt concentration. The plants under salinity condition change their metabolism to overcome the changed environmental condition. According to the SP content decreased on account of salinity stress, one of the mechanisms affected by salt stress



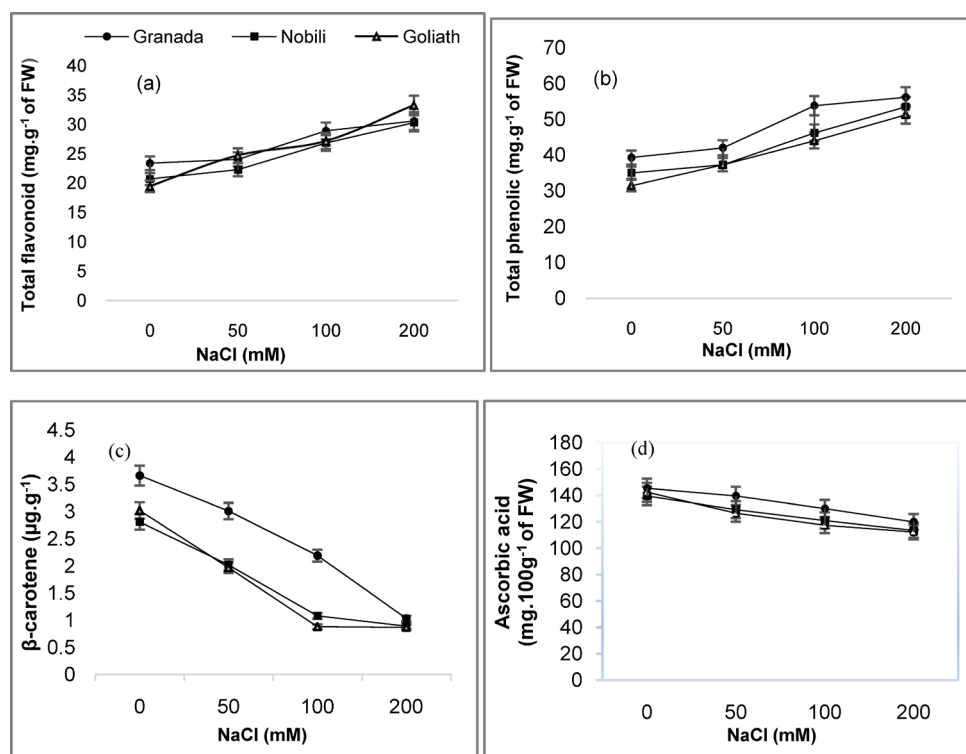


**Figure 1.** Effect of salt on accumulation of organic compound in pepper cultivars (90 DAP). Proline content (a), soluble proteins (b), fructose content (c) and glucose content (d). Bars are means ( $n = 5$ )  $\pm$  SD.

in plants was protein synthesis [47]. Proline, which is an amino acid is one such organic solute that plays a major role in this osmotic adjustment [48]. Proline is one of well-known osmoprotectants and its accumulation is widely observed in various organisms under salt stress. The amino-acid may play a role in protecting membranes and proteins against adverse effects of higher concentrations of inorganic ions and temperature extremes. Salinity treatments caused the increased PRO content in pepper plant [48]. One mechanisms utilized by the plants for overcoming the salt stress effects might be via accumulation of compatible osmolytes, such as proline and soluble sugar. Great diversity of free sugars within the *Capsicum chinense* gene pool [49]. [50] confirmed that tomato (*Lycopersicon esculentum*. Pepe), total fruit sugar content increased with increased salinity; sucrose played a main role in the regulation of the root osmotic potential followed by K, GLU and Na this agree with the results [51]. The reduction of sugars content in pepper fruit with salinity could be due to this increase in fruit respiration. The accumulation of osmolyte compounds is often proposed as a solution to overcoming the negative consequences of water deficits in crop production which has been proposed as an adaptive mechanism for drought and salt tolerance. Indeed, osmolyte accumulation in plant cell results in a decrease of the cell osmotic potential and help in the maintenance of water absorption and cell turgor pressure, which might contribute to sustaining physiological processes, such as stomatal opening, photosynthesis and expansion growth [52].

### 3.3. Antioxidant Compound

NaCl effect on fruits TF, TP, CA and ASA concentrations is shown in **Figure 2**. A significant increase ( $p < 0.05$ ) was observed for TF and TP at 100 and 200 mM NaCl in “Granada” and “Nobili” and 50 mM NaCl in “Goliath”. These increases for TF were 30.72% in “Granada”, 46.59% in “Nobili” and 71.06% in “Goliath”; and 42.89% in “Granada”, 52.74% in “Nobili” and 63.13% in “Goliath” for TP under the highest NaCl (200 mM) in comparison with the control (**Figure 2(a)** and **Figure 2(b)**). Salt effect resulted in a significant decrease for ASA and CA ( $p < 0.05$ ). The decreases for ASA were 79.83% in “Granada”, 82.71% in “Nobili” and 82.58% in “Goliath”; and 27.87% in “Granada”, 30.60% in “Nobili” and 31.45% in “Goliath” for CA under the highest NaCl (200 mM) in comparison with the control (**Figure 2(c)** and **Figure 2(d)**). In other vegetables such as amaranth species, [53] observed a decrease of ascorbic acid content with increase of salt concentration. Salinity decreased the ASA content of pepper fruits, and this effect was dependent on the maturity stage [54]. In addition, the possibility for a plant to limit salt accumulation within its tissues triggers differences in the intensity of salinity stress perceived by the plant. Furthermore, it is well known that ASA is an important component of several fruits (tomato, pepper, and strawberry) that reacts with singlet oxygen and other free radicals and suppresses peroxidation [55]. In tomato fruits, the increase of ascorbic acid contents

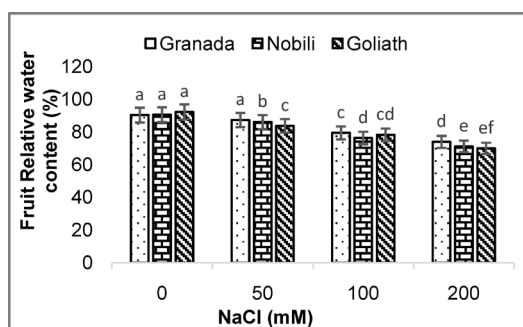


**Figure 2.** Effect of salt on antioxidant compounds in pepper fruit cultivars at the mature stage (90 DAP). Total flavonoid content (a), total phenolic content (b),  $\beta$ -carotene content (c) and ascorbic acid content (d). Bars are means ( $n = 5$ )  $\pm$  SD. Means followed by different letter are significantly different ( $p < 0.05$ ).

under salt stress was reported [41]. Carotenoids are widely known as powerful natural antioxidants that act as the most efficient singlet oxygen quenchers *in vitro* among common carotenoids ([56]). In agreement with these data, [57] showed that CA in tomato fruit was significantly decreased under salt stress. Thus, under the prevailing experimental conditions the decrease in CA contents may relate to the decrease in photosynthetic processes under salinity. A possible explanation would be that salinity may inhibit or upregulate the biosynthetic pathway of carotenoids via inhibition of the genes encoding enzymes related to  $\beta$ -carotene [58]. Salt stress caused an inhibition in the expression of the gene encoded for lycopene  $\beta$ -cyclase, the enzyme that converts lycopene to beta carotene [59]. The results on phenol contents are in conformity with the findings in pepper [54] and tomato fruits [60], while it contrasts with those of [61] in other tomato varieties. In addition, [62] reported that adding NaCl to the nutrient solution did not affect phytonutrients such as flavonoids (quercetin). It is well known that anthocyanins are members of the flavonoid class of plant secondary metabolites that are not usually synthesised in tomato fruits [63]. The increased synthesis of TP, TF contents under saline conditions may reflect some kind of defense against stress conditions since salt stress was accompanied by increased production of reactive oxygen species [64].

### 3.4. Fruit Relative Water Content

Fruit RWC of pepper cultivars at different salinity levels is depicted in **Figure 3**. There are significant differences between cultivars. A significant decrease in RWC was found at high salinity level (200 mM) in all cultivars compared with control. This decrease may be due to the reduction in water uptake [65] and/or its harmful effect on cell wall structure [66]. The ameliorative effects of these treatments on RWC could be due to the increase in osmoregulators, as well as to osmotic adjustment in plant cells [67] [68]. So, increasing NaCl salinity concentration tended to reduce the absorption of water leading to a drop in water content, the inhibitory effect of NaCl on growth parameters could be attributed to the osmotic effect of NaCl salinity, in addition, the changes in water status under NaCl stress may cause a reduction in meristem activity as well as cell elongation [48].



**Figure 3.** Effect of salt on fruit relative water content in pepper varieties at vegetative mature (90 DAP). Bars are means ( $n = 5$ )  $\pm$  SD Means followed by different letter are significantly different ( $p < 0.05$ ).

### 3.5. Minerals

Pepper fruit contains many essential minerals. Most minerals showed variation among the 03 pepper varieties at the different levels of NaCl. Salt treatments differently affected the fruit contents of P, Na, Mg, Ca, S, Fe, Cu, Zn and Mn, depending on the genotype (Table 2(a) and Table 2(b)). The main effect of NaCl on fruit Na concentrations of plants under salt stress showed significant increases as compared to control plants (Table 2(a)). The highest Na concentrations (11.22 mg on 100 g) were detected in “Goliath” cultivar while the lowest (10.62 mg on 100 g) were recorded in “Granada” at 200 mM NaCl (Table 2(a)). In this study, K, Ca, P, Mn, S, Cu, Fe, Zn and Mg concentrations were significantly reduced with increasing salinity in all cultivars (Table 2(a) and Table 2(b)). K is the most abundant mineral, followed by P, Mg, Ca, S, Fe, Cu, Zn and Mn. The levels of each mineral across four levels of NaCl showed a high (significant) variation, indicating these compounds are strongly influenced by salinity. The Ca, Mg, Na, Cu, Fe, P, S, Zn, Mn, K content decreased significantly between 100 and 200 mM NaCl in “Granada” and “Nobili” cultivars and 50 mM NaCl in “Goliath” cultivar (Table 2(a) and Table 2(b)). According to the analysis of variance

**Table 2.** (A) Effect of salt stress on ions concentrations (mg on 100 g of fresh weight) of pepper fruit cultivars at mature stage (90 DAP); (b) Effect of salt stress on ions concentrations ( $\mu\text{g}$  on 100 g of fresh weight) of pepper fruit cultivars at mature stage (90 DAP).

(a)							
Cultivar	Treatment (mM NaCl)	Na	K	Ca	Mg	P	S
Granada	0	4.88 ± 0.01 <sup>bc</sup>	263.71 ± 2.43 <sup>a</sup>	12.89 ± 0.21 <sup>a</sup>	16.81 ± 0.34 <sup>a</sup>	36.81 ± 0.39 <sup>a</sup>	12.39 ± 0.31 <sup>a</sup>
	50	5.37 ± 0.04 <sup>b</sup>	257.57 ± 2.47 <sup>b</sup>	13.97 ± 0.23 <sup>a</sup>	15.12 ± 0.37 <sup>a</sup>	32.34 ± 0.41 <sup>b</sup>	10.86 ± 0.29 <sup>a</sup>
	100	8.14 ± 0.07 <sup>a</sup>	222.72 ± 2.49 <sup>e</sup>	11.39 ± 0.19 <sup>a</sup>	13.99 ± 0.24 <sup>ab</sup>	29.33 ± 0.44 <sup>bc</sup>	11.53 ± 0.25 <sup>a</sup>
	200	10.62 ± 0.08 <sup>a</sup>	201.26 ± 1.83 <sup>f</sup>	11.77 ± 0.25 <sup>a</sup>	11.30 ± 0.42 <sup>b</sup>	28.59 ± 0.42 <sup>c</sup>	8.63 ± 0.23 <sup>b</sup>
Nobili	0	5.39 ± 0.03 <sup>b</sup>	243.19 ± 2.39 <sup>d</sup>	13.27 ± 0.125 <sup>a</sup>	15.57 ± 0.39 <sup>a</sup>	33.53 ± 0.38 <sup>ab</sup>	11.69 ± 0.19 <sup>a</sup>
	50	6.46 ± 0.05 <sup>b</sup>	221.47 ± 2.42 <sup>e</sup>	10.82 ± 0.26 <sup>ab</sup>	16.19 ± 0.36 <sup>a</sup>	34.80 ± 0.36 <sup>a</sup>	9.31 ± 0.18 <sup>ab</sup>
	100	8.80 ± 0.06 <sup>a</sup>	205.79 ± 3.44 <sup>f</sup>	11.43 ± 0.19 <sup>a</sup>	12.82 ± 0.41 <sup>b</sup>	31.09 ± 0.32 <sup>b</sup>	7.62 ± 0.21 <sup>b</sup>
	200	10.88 ± 0.09 <sup>a</sup>	190.15 ± 2.51 <sup>g</sup>	9.93 ± 0.22 <sup>c</sup>	10.91 ± 0.39 <sup>bc</sup>	27.15 ± 0.38 <sup>c</sup>	8.35 ± 0.18 <sup>b</sup>
Goliath	0	5.15 ± 0.10 <sup>b</sup>	251.81 ± 2.42 <sup>c</sup>	12.81 ± 0.19 <sup>a</sup>	14.94 ± 0.37 <sup>a</sup>	30.26 ± 0.42 <sup>bc</sup>	11.90 ± 0.23 <sup>a</sup>
	50	7.41 ± 0.08 <sup>ab</sup>	218.53 ± 1.44 <sup>e</sup>	10.26 ± 0.21 <sup>a</sup>	11.92 ± 0.29 <sup>b</sup>	31.42 ± 0.44 <sup>b</sup>	8.92 ± 0.22 <sup>b</sup>
	100	9.64 ± 0.07 <sup>a</sup>	192.45 ± 2.47 <sup>g</sup>	8.88 ± 0.26 <sup>c</sup>	10.28 ± 0.24 <sup>bc</sup>	23.77 ± 0.41 <sup>d</sup>	7.41 ± 0.21 <sup>b</sup>
	200	11.22 ± 0.09 <sup>a</sup>	187.68 ± 1.54 <sup>h</sup>	8.27 ± 0.23 <sup>c</sup>	8.12 ± 0.28 <sup>c</sup>	18.20 ± 0.39 <sup>e</sup>	6.67 ± 0.19 <sup>bc</sup>
Two way ANOVA results							
Cultivar (C)		*	*	NS	*	*	NS
Salt treatment (S)		**	*	*	*	*	*
Interaction C X S		*	*	NS	NS	*	NS

Values shown are means (n = 5) ± SD; within columns, means followed by different letter are significantly different (p < 0.05). \*\*, \* significant at 1% and 5% probability levels, respectively, NS not significant.

(b)

Cultivar	Treatment (mM NaCl)	Mn	Cu	Fe	Zn	K/Na	Ca/Na	Mg/Na
Granada	0	201.1 ± 4.21 <sup>a</sup>	141.31 ± 3.28 <sup>b</sup>	887.66 ± 5.27 <sup>a</sup>	572.22 ± 2.99 <sup>a</sup>	54.03 <sup>a</sup>	2.64 <sup>a</sup>	3.44 <sup>a</sup>
	50	198.91 ± 3.49 <sup>b</sup>	125.23 ± 3.09 <sup>d</sup>	855.32 ± 4.88 <sup>b</sup>	537.52 ± 3.02 <sup>c</sup>	47.96 <sup>b</sup>	2.60 <sup>a</sup>	2.81 <sup>a</sup>
	100	158.84 ± 3.38 <sup>c</sup>	111.12 ± 2.89 <sup>e</sup>	818.21 ± 5.52 <sup>c</sup>	511.23 ± 3.33 <sup>e</sup>	27.36 <sup>c</sup>	1.39 <sup>a</sup>	1.71 <sup>a</sup>
	200	139.71 ± 3.66 <sup>e</sup>	107.09 ± 3.02 <sup>f</sup>	790.33 ± 3.29 <sup>f</sup>	499.93 ± 3.71 <sup>h</sup>	18.95 <sup>d</sup>	1.11 <sup>ab</sup>	1.06 <sup>b</sup>
Nobili	0	177.48 ± 4.09 <sup>c</sup>	140.25 ± 3.13 <sup>b</sup>	801.27 ± 3.67 <sup>e</sup>	558.02 ± 2.96 <sup>b</sup>	45.11 <sup>b</sup>	2.46 <sup>a</sup>	2.88 <sup>a</sup>
	50	163.26 ± 4.27 <sup>d</sup>	133.20 ± 2.33 <sup>c</sup>	788.04 ± 4.21 <sup>e</sup>	524.71 ± 3.02 <sup>d</sup>	34.28	1.67 <sup>a</sup>	2.50 <sup>a</sup>
	100	128.87 ± 4.11 <sup>h</sup>	127.11 ± 2.67 <sup>d</sup>	735.19 ± 4.44 <sup>h</sup>	501.88 ± 3.66 <sup>e</sup>	23.38 <sup>c</sup>	1.29 <sup>ab</sup>	1.45 <sup>a</sup>
	200	120.73 ± 3.21 <sup>i</sup>	110.08 ± 3.01 <sup>e</sup>	709.81 ± 3.89 <sup>j</sup>	488.05 ± 3.43 <sup>i</sup>	17.47 <sup>d</sup>	0.91 <sup>b</sup>	1.01 <sup>b</sup>
Goliath	0	180.91 ± 4.55 <sup>c</sup>	145.29 ± 3.29 <sup>a</sup>	813.40 ± 4.10 <sup>d</sup>	537.79 ± 3.61 <sup>c</sup>	48.89 <sup>b</sup>	2.48 <sup>a</sup>	2.90 <sup>a</sup>
	50	157.75 ± 3.89 <sup>e</sup>	133.11 ± 2.58 <sup>c</sup>	761.11 ± 3.77 <sup>h</sup>	509.72 ± 3.80 <sup>f</sup>	29.49 <sup>b</sup>	1.38 <sup>a</sup>	1.60 <sup>a</sup>
	100	143.61 ± 3.30 <sup>f</sup>	112.26 ± 2.17 <sup>e</sup>	723.78 ± 3.22 <sup>i</sup>	480.69 ± 2.76 <sup>j</sup>	19.96 <sup>d</sup>	0.92 <sup>b</sup>	1.06 <sup>b</sup>
	200	129.71 ± 2.99 <sup>h</sup>	101.07 ± 2.15 <sup>e</sup>	694.25 ± 3.19 <sup>k</sup>	444.58 ± 2.88 <sup>k</sup>	16.72 <sup>de</sup>	0.73 <sup>bc</sup>	0.72 <sup>bc</sup>
Two way ANOVA results								
Cultivar (C)		*	*	*	*	*	NS	*
Salt treatment (S)		**	*	**	*	*	*	*
Interaction C X S		*	*	*	*	NS	NS	NS

Values shown are means (n = 5) ± SD; within columns, means followed by different letter are significantly different (p < 0.05). \*\*, \* significant at 1% and 5% probability levels, respectively, NS not significant.

of the mineral nutrient contents in the sweet pepper fruits, the interaction of the NaCl with the cultivars affected their Ca, P, Zn, Fe, Mn, S, K, Na and Mg contents (**Table 2(a)** and **Table 2(b)**). The cultivars factor had a significant effect for all evaluated nutrients. The fruit K/Na, Ca/Na and Mg/Na ratios were found to be significantly highest in “Granada” and lowest in “Goliath” (**Table 2(b)**). The content of K, Ca and Mg in *Zea mays* plant decreased significantly under salinity stress, compared with control [69]. The deleterious effects of salinity on plant growth are associated with low water potential of the root medium which causes a water deficit within the plant; toxic effects of ions mainly Na<sup>+</sup> and Cl<sup>-</sup>; nutritional imbalance caused by reduced nutrient (K, S, P, Mg, Cu, Fe, Ca, Mn and Zn) uptake and/or transport to the shoot. Salinity mainly causes both hyper-osmotic stress and hyper-ionic toxic effects and the consequence can be plant demise [70]. The contents of K in the fruit tissues decreased with increasing salinity for all varieties, confirming the effect of salt stress by the high concentration of Na [71]. Potassium may play a role in the synthesis of endogenous plant hormones [72]. Despite its obvious importance, the low mobility of Ca<sup>2+</sup> make the rates of its uptake and distribution limiting processes for many key plant functions. Furthermore, the general lack of recognition of the limiting role of Ca<sup>2+</sup> is due in part to the fact that some important plant functions are controlled by changes in very small physiologically active pools of Ca<sup>2+</sup> within the

cytoplasm. As such, whole-leaf  $\text{Ca}^{2+}$  levels might not reflect any potential limitations [73]. [74] found that the P content in tomato (*Lycopersicon peruvianum* L.) plant decreased was increased NaCl at 150 mM. Decreased P contents due to increasing salinity were caused probably by the high levels of the Cl ion, which can have antagonistic interactions with phosphorus [75], however, there is no clear evidence of the interaction between salinity and changes in P absorption [71]. Salt stress significantly increased the sodium content of pepper fruit. In agreement with these data, several authors reported that salt stress induced the accumulation of  $\text{Na}^+$  in pepper fruit, and this may also result in an enhancement of oxidative parameters [41]. In this study, K, Ca, Cu, Mn, Fe, Zn, S and Mg concentrations were significantly reduced with increasing salinity in all cultivars. It has been reported that salinity affects plant physiology through changes of water and ionic status in the cells because of ionic imbalance due to excessive accumulation of Na and Cl and reduced uptake of other mineral nutrients, such as K, Ca and Mg [70]. This could be also attributed to the competition of Na with the uptake K, Ca, Mg resulting in a K/Na, Ca/Na and Mg/Na antagonism [76]. The fruit K/Na, Ca/Na and Mg/Na ratios were found to be significantly highest in “Granada” and lowest in “Goliath” (Table 2(b)). The relationship between the degree to which plant tolerate salt stress and their capacity to maintain a high leaf ratio K/Na has been noted by several authors [77]. The levels of each mineral across levels of NaCl showed a high (significant) variation, indicating these compounds are strongly influenced by salinity. Previous studies have shown that amounts of minerals in pepper fruit depend on the ripening stage, agricultural practices, genotype and environmental factors [78] [79].

#### 4. Conclusion and Recommendations

Salinity negatively affects pepper organic and inorganic compounds, antioxidant activity and ASA content and yield, while improving fruit quality. Results from this investigation show that mineral nutrients, agro-morphological, osmolytes and antioxidant compounds to salt stress response among pepper varieties exist. The results obtained show that, the increasing within twelve weeks of treatment doses of NaCl, inhibited all agro-morphological parameters, acid ascorbic,  $\beta$ -carotene and inorganic compound (Cu, P, Mn, S, Fe, K, Zn, Ca and Mg) in fruit from 50 mM NaCl for Goliath variety and from 100 to 200 mM NaCl, for Granada and Nobili varieties and the total flavonoid, phenolic, fructose, glucose, proline, soluble protein and  $\text{Na}^+$  significant accumulation in the fruits. The good behaviour of Granada variety in the face of salinity can be considered for their use to better enhance the Sahelian and coastal areas.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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## Abbreviations

Calcium-Ca;  $\beta$ -carotene-CA; chlorophyll-CHL; days after planting-DAP; flavonoids-FLA; magnesium-Mg; Ascorbic acid-ASA; fructose-FRU; glucose-GLU; number of ripe fruit per plant-NF; fruit length-FL; fruit diameter-FD; sodium-Na; zin-Zn; iron-Ir; Manganese-Mn; sulfate-S; phosphorus-P; potassium-K; proline-PRO; relative water content-RWC; fruit dry weight-FDW; fruit fresh weight-FFW; thickness of fruit-TF soluble proteins-SP; total free amino acids content-FAA; copper-Cu; iron-Fe; total phenolic-TP; total soluble sugars-SS