

EFFECTS OF SALINITY ON GROWTH, PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF TOMATO

Nasratullah Habibi^{1,2}, Naveedullah Sediqui^{1,3},

Naoki TERADA¹ Atsushi SANADA¹, and Kaihei KOSHIO¹

¹Graduate School of Agriculture, Tokyo University of Agriculture,
1-1-1 Sakuragaoka, Setagaya-ku, Tokyo Japan 156-8502

²Faculty of Agriculture, Balkh University, Balkh 1701, Afghanistan.

³Faculty of Agriculture, Alberoni University, Kapisa 1701, Afghanistan.

Corresponding author: nt204361@nodai.ac.jp

(Received: May 18, 2021; Accepted: August 31, 2021)

Salinity stress creates serious problems for tomato production in dry climate regions like Southeast Asia. Two sets of experiments were conducted, where the initial experiment was on effects of salinity on seed germination and seedling characteristics in the laboratory and the second experiment was to evaluate the effects of salinity on growth, physiological and biochemical responses of tomato. These experiments were carried out in the greenhouse of the Laboratory of Tropical Horticulture Science in Tokyo University of Agriculture during 2020 and 2021. Tomato (*Solanum lycopersicum* L. cv. Micro-Tom) was used as plant material and sodium chloride was used as salt. The experiment was designed in complete randomized design with 4 salt treatments (50, 100, 150, and 200 mM), and control (no sodium chloride). In the initial experiment, it was found that the germination rate, shoot and root length were significantly reduced under saline conditions. In the second experiment, the seeds were first grown in vermiculite, and 3-4 leaf seedlings were transplanted into a hydroponics system where the saline treatments were applied. It was observed that salinity, at the rates tested, decreased plant height, root length, the number of flowers, photosynthetic rate, transpiration rate, and stomatal conductance, but it increased leaf temperature. Moreover, sugars decreased under salinity, while organic acids, MDA and proline content increased. Proline and MDA are produced in response to salt stress. Accordingly, fruit yield was reduced under salinity as compared to control.

Key words: hydroponics, photosynthetic rate, sugars, organic acid, malondialdehyde (MDA), and proline.

INTRODUCTION

Tomato (*Solanum lycopersicum*) is a vegetable with immense economic importance and is grown worldwide (Saito et al. 2011). The demand for tomatoes is increasing with the rapid increment in population, and in the near future, the demand for tomato will increase while there will be low production (Hernández-Pérez et al. 2020) due to land unavailability, and stagnation in yield due to biotic, and abiotic stress factors which are the main constraints for tomato producers around the globe. It is estimated that by the year 2050, salt stress alone will cause a 50% loss in yield of vegetables including tomato (Wang et al. 2003), and it will be a huge concern for southeast Asian countries.

The negative effect of salt stress on tomatoes is a result of retarded plant growth due to decline in photosynthetic rate (Giannakoula and Ilias 2013), which leads to a reduction in fruit size and total yield per plant, which are the most important factors for tomato producers. Moreover, in the case of growth, it has been reported that tomato plants under salinity stress have shorter plant height and

lower leaf area. Also, leaf relative chlorophyll content (SPAD value) which shows the amount of nitrogen, the key element for enhancement of photosynthesis, could also be affected under saline conditions (Zhang et al. 2017). Tomato seeds are very sensitive to salinity as germination and vigor can be compromised due to small amounts of salt. Additionally, salinity is supposed to decelerate the growth of tomato seedlings at the initial growth stage (Singh et al. 2012). Plant roots become shorter and the ability to absorb water and nutrients decreases under salinity and it leads the plant to be stunted. Therefore, a disruption of photosynthesis in leaves will occur and there will be shortage of water in leaves for transpiration and cooling (Ismail et al. 1994). Furthermore, if leaf temperature increases, plants start to produce chemical compounds such as proline and malondialdehyde (MDA) to combat the shock, and electrons start to leak from the cells (Gharsallah et al. 2016). Interestingly, salinity in a small amount somehow can improve the fruit quality, and the reason is due to the concentration of sugars in small fruits. However, fruit quality in plants grown under high salinity is still questionable (Zhu et al. 2018). Plants grown under salinity, produce fruits with higher organic acids even in pre and post-harvest stages (Zushi and Matsuzoe 2006). Currently, in many countries people use the green tomatoes with high organic acids to make pickle and serve it as food supplement (Locato et al. 2013), and people in developed countries like Japan appraise fruit quality and like to buy fruits with higher Brix, sugar content, and superior taste (Johkan et al. 2014; Saito and Matsukura 2015).

Therefore, the current study was carried out to explore the effects of salinity on (a) germination and seedling growth of tomato, (b) growth, physiological characteristics and yield of tomato, and (c) biochemical attributes of tomato fruits.

MATERIALS AND METHODS

Two experiments were conducted from 2020 to 2021 to investigate tomato response under saline conditions in both seedling and fruit bearing stages. The seedling experiment was done in the laboratory of Tropical horticultural science from June to July 2020, while the second one was carried out from October 2020 to March 2021 under greenhouse conditions in the green house belonging to Tropical Horticultural Science laboratory, Tokyo University of Agriculture, Japan. Tomato (*Solanum lycopersicum* L. cv. Micro-Tom) seeds were used as plant material and sodium chloride as salt.

In the first experiment, tomato seeds were subjected to germination in Petri dishes under normal and saline conditions and placed in a growth chamber (CHF-405 Cultivation Chamber, Japan) to determine percent of germination and seedling growth. In each treatment, 100 seeds were used, and the experiment was designed in a complete randomized design (CRD) with three replications. The daily light was adjusted for 12 hours, and the temperature was set up at 25 °C. Observation was done daily, and watering done when needed. In the second experiment, Micro-Tom seeds were cultivated first in rockwool under normal conditions, then at 3-4 leaf stage, transplanted to a hydroponics system in the greenhouse, and the salinity treatments were applied. The hydroponics system was set using solution reservoir separately for each treatment, a cork board to keep the plants on the solution and a pump for aeration once in 7:00 – 8:00 AM and once 04:00 – 05:00 PM. For hydroponics solution, tap water was used. OAT House 1 and 2 fertilizers (OAT Agrio Co., Ltd Japan) were used for making the hydroponics nutrient solution, while according to OAT Agrio company recommendation, OAT 1 (OAT green) was used 1 kg / 1000 liters of water and OAT 2 (OAT blue) as 1.5 kg / 1000 liters of water. The OAT House 1 contains 11 % NO₃-N and 16.4 % calcium. The OAT House contains 10 % nitrogen (N), 8 % phosphorus (P), 27 % potassium (K), 4 % magnesium (Mg), 0.10 % manganese (Mn), 0.18 % iron (Fe), 0.002 % copper (Cu), 0.006 % zinc (Zn), and 0.002 % molybdenum (Mo).

Salt stress. Salinity treatments were set as: Control (no NaCl), T1 (50 mM NaCl), T2 (100 mM NaCl), T3 (150 mM NaCl), and T4 (200 mM NaCl). In experiment 1, saline solution was applied (according to mM) every two days. In experiment 2, tap water was used for irrigation, and its pH was adjusted

between 5.8 – 6 using sodium hydroxide and hydrochloric acid. Then NaCl was added to the hydroponics solution for the appropriate molarity, mixed well and used for irrigation.

Parameters. In experiment 1, germination capacity was measured using the method by Bam et al. (2006). The relative injury rate in seeds was measured using the formula described by Fetouh and Hassan (2014).

$$\text{Relative Injury Rate (RIR)} = (\text{GC \%} - \text{GS\%})/\text{GC\%} \dots\dots\dots 1$$

In formula 1, GC % represents the germination percentage in control plants, and GS% represents the germination percentage in salt treatment.

Root and shoot length was measured using ImageJ software after scanning the seedlings using a digital scanner (EPSON DS-G2000, Japan). Seedling vigor index (Masuthi et al. 2015) and seedling height reduction (Kandil et al. 2012) were calculated using the following formula:

$$\text{Seedling vigor index (SVI)} = \text{L/G \%} \dots\dots\dots 2$$

$$\text{Seedling height reduction (SHR)} = (\text{LC-LS})/\text{LC} \times 100 \dots\dots\dots 3$$

In formula 2, L represents the seedling length (cm), and G% represents the germination percentage. In formula 3, LC represents seedling length in control, and LS represents seedling length in salt treatment.

Growth, yield and physiological traits. Among the growth parameters, plant height and root length were measured once a week for 6 weeks starting at first flowering. The number of flowers per plant was counted and recorded weekly from the first flowering and continued for five weeks till the end of the flowering stage. Photosynthetic rate, transpiration rate, stomatal conductance, and leaf temperature were measured using an LCi-SD Portable Photosynthesis System (ADC Bioscientific, Hoddesdon, UK) at the fruit-bearing stage. Yield and its components like individual fruit weight, number of fruits per plant, and total yield per plant were recorded when the fruits ripened.

Leaf area measurement. Leaf area was measured using ImageJ software after scanning the leaves using a digital scanner (EPSON DS-G2000, Japan). Five leaves from each treatment were taken for leaf area measurement, and the data recorded for further analysis.

Electrolyte leakage. Electrolyte leakage (EL) was measured at flowering and fruit-bearing stages based on the method described by Hatsugai and Katagiri (2018). Fifteen leaves from each treatment were cut by a 1-cm diameter stainless steel cork borer and kept inside pure water in 2 ml tubes under $25 \pm 1^\circ\text{C}$ for 20 minutes. An electrical conductivity meter (LAQUATWIN-S070, Horiba Scientific Ltd., Japan) was used to measure EC and the reading was recorded as EC1. Then the tubes were put in the water bath for 20 minutes under 70°C . Then, cooled at room temperature and again EC was measured and recorded as EC2. Therefore, EL was calculated using the below formula:

$$\text{Electrolyte leakage (\%)} = (\text{EC1}/\text{EC2}) \times 100 \dots\dots\dots 4$$

Sugars, and organic acid analysis. Sugar contents of tomato fruits (glucose, fructose, and sucrose) were measured with Shimadzu HPLC 2007 system. The amount of sugars were quantified using the method described by Agius et al. (2018). Mature tomato fruits were harvested, crushed in liquid nitrogen and 200 mg of the powder was placed in a 2 ml plastic vials and 1.8 ml of 5% aqueous ethanol was added. The samples were vortexed and centrifuged at 15000 rpm for 15 minutes at 4°C . From the supernatant, 900 μl was collected and a 900 μl of acetonitrile was added, vortexed and poured into a 2.5

ml syringe equipped with polytetrafluoroethylene (PTFE) 0.20 µm syringe filter unit. The filtrate samples were placed into 2 ml HPLC vials and analyzed for sugars and organic acids by HPLC.

Citric acid and malic acid were measured by ion chromatography following the method described by Agius et al. (2018) with some modifications using a Shimadzu 10AVP (Japan) HPLC fitted with a column series SCR-102H×2 (length 8 mm × 300 mm × 2), at 40 °C and coupled with a Shimadzu CDD 10AVp conductivity detector. These measurements were made under circulation of a mobile phase composed of 100% 3mM HClO₄ at a flow rate of 0.8mL/min. Tomato fruit powder (200 mg) was mixed with 1.8 ml ultra-pure water, vortexed and centrifuged at 15000 g for 15 minutes at 4 °C. The supernatant was collected in a 2 ml plastic tube and transferred into a 2.5 ml syringe equipped with a 0.45 µm DISMIC cellulose acetate syringe filter, manufactured by membrane solutions Co. Ltd (Japan). The filtrate samples were poured into 2 ml glass vials for HPLC analysis of organic acids.

Fruit brix was measured by slicing tomato fruits and removing the jelly layer and seeds. The pulp was crushed, and the juice was filtered by two-layer mesh cloth. Brix was measured from the juice using an Asone refractometer and the amount was presented as percentage (%).

Color measurements. Fruit color was quantified using a Handy Colorimeter (NR-3000, Nippon Denshoku Ind., Ltd., Japan). The method used was CIE 1976 L*a*b* method (Anon. 1974, Kuehni 1976). The data was given as numerical a, b and L values separately. The maturity stage was decided by the color of control (no salinity) plants and as a-color shows redness, therefore we made our decision about fruit maturity by considering a-color (Kuehni 1976).

Ethylene production measurement. The fruit weight was measured, and the fruits were kept in 550 ml jars under a black cloth. After one hour, 1 ml gas from the jars was taken by plastic syringe and injected into the GC-FID (Gas Chromatograph-Flame Ionization Detector, GC-14B Japan) with the following parameter: column temperature = 80 °C, injector temperature = 180 °C and detector temperature = 200 °C. The GC column Sunpak-A (Shinwa chemical industries, Japan) was used for ethylene production measurement. The carrier gas was ultra-high-quality nitrogen, and column pressure was 6 kg cm⁻². The GC reading was then converted to nL g⁻¹ h⁻¹ using the following formula:

$$E = [(MR*V)/W]/h..... 5$$

where E is the amount of ethylene, MR is the GC reading, V is jar volume, W is fruit weight, and h is number of hours of keeping fruits inside jars under a black cloth.

Fruit firmness measurement. A Multilateral Tester model 2519-104 (INSTRON Company), was used to measure fruit firmness when the 1.0 cm diameter plunger pressed tomato fruit at 1 mm/sec speed. Five fruits were measure per treatment.

Malondialdehyde and proline. Malondialdehyde (MDA) was measured according to the method described by Wang et al. (2005) with some modification. Leaf tissue samples (2 g) were collected from each treatment in five replications and homogenized in 6 ml of 100 mM sodium phosphate buffer (pH 6.4) containing 0.5 g polyvinylpolypyrrolidone (PVPP). The homogenized solution was filtered through a cotton cloth, centrifuged at 15,000 rpm for 30 minutes at 4 °C and the supernatant was used directly for assay. The MDA content was determined by adding 2 mL of 0.5 % trichloro-butyric acid (TBA) in 15 % trichloro-acetic acid (TCA) to a 1 mL sample. The mixture was heated at 95 °C using a water bath for 20 minutes and cooled immediately. The absorbance of the supernatant was measured using a spectrophotometer (Hitachi U-1100, Japan) at 532 nm and 600 nm. The level of TBA equivalents (nmol.g⁻¹FW) was equal to $\{[(A532 - A600)/155,000] \times 106\}$.

Proline content was analyzed by the modified procedure of Gharsallah et al. (2016). Ten leaves were taken from each treatment, and approximately 100 mg of the crushed leaves was homogenized with 250 µl each of methanol (analytical reagent grade) and chloroform (analytical reagent grade) and heated in a water bath for 5 minutes at 37 °C. Ribitol solution (50 µl) and pure water (175 µl) were added, and vortexed. All samples were centrifuged for 10 minutes at 140 x 100 rpm, and 80 µl of the supernatant from each sample was vaporized using vaporizer, and was placed in a freeze dryer for 24 hrs. A solution of 20 mg of methoxamine hydrochloride and 1 ml of pyridine was prepared and 40 µl of the prepared solution was added to each sample and was heated at 37 °C for 90 minutes. Finally, 50 µl of MSTFA solution was added to each sample and analyzed for proline using a gas chromatograph-mass spectrophotometer (GCMS-QP2010 Plus Shimadzu, Japan). The amount of free proline was quantified using a standard curve and expressed as µmole g⁻¹ tissue fresh weight.

Statistical analysis. The experiment was laid out as a completely randomized design. The data were analyzed with analysis of variance (ANOVA), Pearson's correlation analysis, and the principal component analysis (PCA) method with language R 3.6.2 statistical software and data visualization by Python 3.7.4 (Jupyter Notebook, <https://api.anaconda.org>). The means were compared using Tukey's test at 0.05 level.

RESULTS AND DISCUSSION

Germination characteristics and seedling growth. Salinity stress caused severe injury to tomato seeds by decreasing germination and increased relative injury rate (Table 1). Likewise, seedling vigor index and seedling height were significantly reduced under saline conditions. About 91.4 % of relative injury rate was observed in high salinity (T4) while it was 0 % in control. Also, a significant seedling height reduction was observed in salinity treatments as compared to control.

Table 1. Germination characteristics and seedling growth parameters.

Treatments	Germination (%)	RIR (%)	SVI	SHR
Control	97.67 ± 1.52 a	0.00 ± 0.00 e	0.04 ± 0.006 c	0 ± 0.00 e
50 mM	84.33 ± 2.76 b	13.65 ± 2.93 d	0.02 ± 0.002 d	0.43 ± 0.03 d
100 mM	31.67 ± 4.04 c	67.57 ± 4.56 c	0.04 ± 0.005 c	0.55 ± 0.05 c
150 mM	15.83 ± 2.25 d	83.79 ± 6.83 b	0.06 ± 0.003 b	0.64 ± 0.04 b
200 mM	8.33 ± 3.35 e	91.47 ± 8.44 a	0.09 ± 0.007 a	0.73 ± 0.06 a
	***	***	**	**

RIR: Relative injury rate, SVI: Seedling vigor index, SHR: Seedling height reduction. Data is represented as 'mean ± SD' and different letters are shown according to the Tukey test at the 0.05 level. The asterisks show significant differences at ***p<0.001, **p<0.01 levels respectively.

Furthermore, control plants produced the longest seedling root and shoot (Fig. 1), which is consistent with the results of Singh et al. (2012). Furthermore, root and shoot growth was not affected severely in control, 50 mM NaCl, and 100 mM NaCl stress plants, while shoots were severely reduced under 150 mM and 200 mM NaCl stress (Fig. 1).

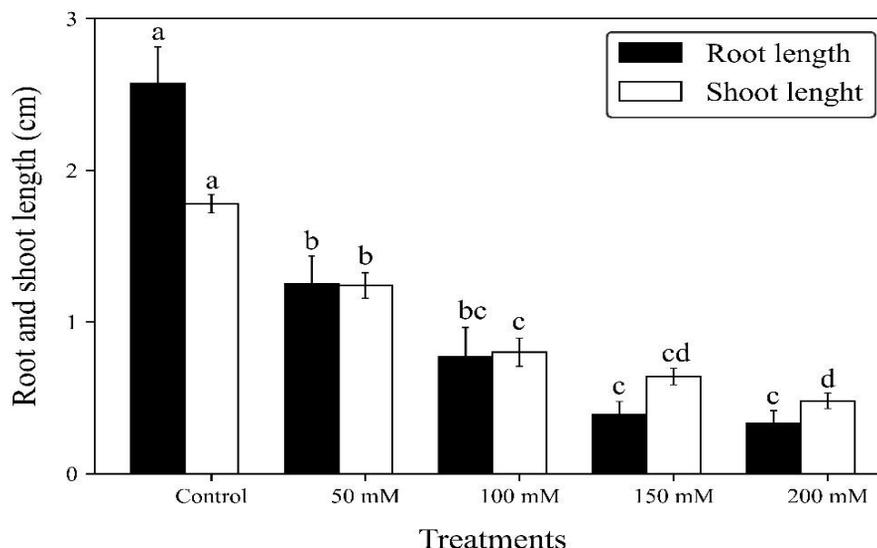


Fig. 1. Effect of salinity on root and shoot length of seedlings. The letters represent significant differences between treatments according to the Tukey test at 0.05 level.

Plant growth parameters. Plant height is the primary attribute that was affected by salinity. There was a significant difference between control and salinity-treated plants. Salinity decreased the root growth and plants produced shorter roots under saline conditions. A similar result was concluded by Ismail *et al.* (1994), that an increase in salinity is followed by a decrease in plant height, and root length. Furthermore, plants under salinity conditions had pale green leaves with lower SPAD values compared to control plants (Table 2). This result agrees with Parvin *et al.* (2015), but with a difference that we used higher concentration of salt.

Table 2. Effects of salt concentration on growth parameters of tomato.

Treatments	Plant height (cm)	Root length (cm)	Leaf area (cm ²)	SPAD value
Control	12.8 ± 1.48 a	17.40 ± 3.91 a	6.40 ± 0.45 a	75.56 ± 7.73 a
50 mM	10.86 ± 0.58 b	16.51 ± 2.31 a	4.26 ± 0.25 b	73.04 ± 6.21 ab
100 mM	8.22 ± 0.84 bc	14.72 ± 1.84 ab	3.00 ± 0.38 c	69.86 ± 3.27 b
150 mM	7.58 ± 1.79 bc	10.60 ± 2.30 bc	2.83 ± 0.27 c	65.98 ± 5.85 bc
200 mM	7.19 ± 0.88 c	8.50 ± 2.69 c	2.32 ± 0.30 c	61.40 ± 7.61 c
	***	*	**	**

The data in the above table are presented as means ± SD, and letters shown according to Tukey test at the 0.05 level. The asterisks show significant differences at ***p<0.001, **p<0.01, *p<0.05 levels respectively.

Moreover, salinity harmed flower production. Control plants had a higher rate of flower production compared to salinity-treated ones. Significant difference was observed only at two weeks while highly significant difference was observed at four weeks (Fig. 2). Similarly, Umar *et al.* (2018) indicated a decreased vegetative growth and number of flowers in tomato under salinity.

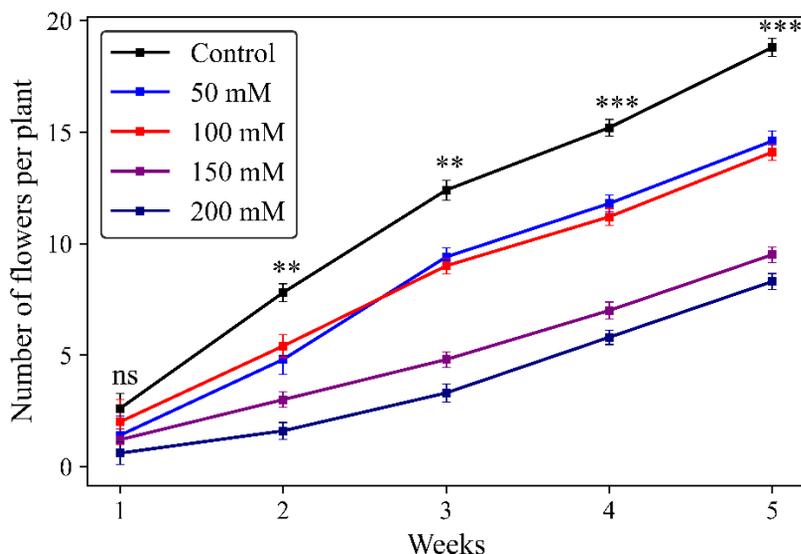


Fig. 2. Effect of salinity on the number of flowers in tomato plants. The asterisks show significant differences at *** $p < 0.001$, ** $p < 0.01$.

Physiological attributes. Physiological parameters including photosynthetic rate, transpiration rate, stomatal conductance, and leaf surface temperature were highly affected by salinity. Principally, positive relation exists between photosynthetic rate, transpiration rate, and stomatal conductance, but they have a negative correlation with leaf surface temperature. Salinity increased significantly leaf surface temperature in tomato plants while this increment was followed by a decrease in photosynthetic rate, transpiration rate, and stomatal conductance. Obviously, when the transpiration is not normal, the plant cannot cool its canopy, so, the leaf surface temperature will increase. Moreover, stomatal conductance leads the photosynthetic materials to other parts of the plant, and salinity decreased it, therefore, photosynthetic rate also decreased (Table 3). A decrease in photosynthetic rate in tomato leaves under salinity was earlier demonstrated by Yang et al. (2020) and Ullah et al. (2020), but in our experiment we evaluated the photosynthetic rate under 200 mM NaCl which has not been reported previously.

Table 3. Effect of salinity on physiological parameters of tomato leaves.

Treatments	Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	Transpiration rate ($\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	Stomatal conductance ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	Leaf surface temperature ($^{\circ}\text{C}$)
Control	1.85 \pm 0.08 a	0.37 \pm 0.11 a	0.32 \pm 0.05 a	24.70 \pm 1.39 c
50 mM	1.11 \pm 0.32 b	0.31 \pm 0.06 ab	0.22 \pm 0.07 b	26.50 \pm 2.04 b
100 mM	0.85 \pm 0.35 bc	0.21 \pm 0.06 bc	0.13 \pm 0.05 c	27.66 \pm 2.51 b
150 mM	0.53 \pm 0.14 cd	0.19 \pm 0.09 bc	0.05 \pm 0.02 cd	31.44 \pm 0.44 a
200 mM	0.35 \pm 0.10 d	0.09 \pm 0.05 c	0.02 \pm 0.01 d	32.36 \pm 0.40 a
	***	**	***	**

The data in the above table are presented as 'mean \pm SD' followed by letters that show significant differences between treatments according to the Tukey test at 0.05 level. The asterisks show significant differences at *** $p < 0.001$, ** $p < 0.01$.

Principal component analysis revealed that 88.3% of data were in PCA1 and 8% in PCA2, showing a good correlation between photosynthetic rate, stomatal conductance, and transpiration rate, but in contrary with leaf surface temperature (Fig. 3). Control plants had a high photosynthetic rate, transpiration rate, and stomatal conductance, but a lower leaf surface temperature. In contrast, saline-treated plants had a higher leaf surface temperature, while the photosynthetic rate, transpiration rate, and stomatal conductance were low.

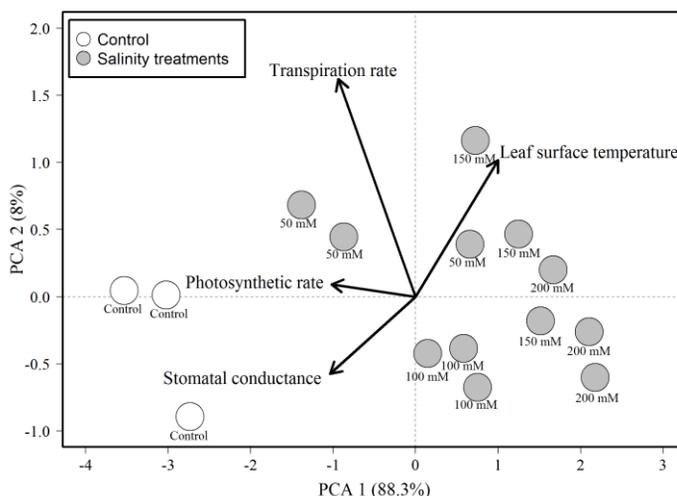


Fig. 3. Principal component analysis of physiological parameters.

Yield and its components. Fruit weight, number of fruits per plant, and yield per plant were highly affected by salinity. Individual fruit weight and the number of fruits per plant were significantly decreased under salinity conditions. There was a significant decrease in yield per plant under NaCl stress treatments as compared to control (Table 4). Salinity stress was observed by Zhang and co-workers (2017) to diminish tomato plant yield under NaCl stress (Control = 0.8 dS m⁻¹ and sodium chloride solution with EC = 2.0 dS m⁻¹) when salt stress was applied separately during the vegetative, flowering and fruiting stages, while we used different salt concentrations starting from transplanting to harvest.

Table 4. Effect of salinity on yield and its components.

Treatments	Fruit weight (g)	Fruits/plant	Yield / plant (g)
Control	3.72 ± 0.74 a	16.0 ± 1.67 a	23.14 ± 3.54 a
50 mM	2.34 ± 0.58 b	9.4 ± 0.84 b	15.87 ± 1.74 b
100 mM	1.57 ± 0.38 bc	5.8 ± 2.26 c	11.52 ± 1.17 c
150 mM	1.28 ± 0.43 c	2.9 ± 0.84 d	10.09 ± 0.86 cd
200 mM	0.83 ± 0.20 c	1.8 ± 0.70 d	6.71 ± 0.53 d
	***	***	***

Yield components are represented as ‘mean ± SD’, and the letters show significant differences between treatments according to the Tukey test at 0.05 level, and the asterisks show significant differences at ***p<0.001, **p<0.01.

Electrolyte leakage and survival rate. Electrolyte leakage shows that electrons are leaked from plant parts as an after effect of injury. In the current experiment, electrolyte leakage increased with the increment of salinity. In the flowering and fruit-bearing stages, a significantly higher electrolyte leakage was observed in saline treatments compared to control. Therefore, the flowers wilted, and less number of fruits were formed in saline treatments. Furthermore, salinity decreased the survival rate (%) in

tomato plants under saline treatments compared to control except for 50 mM NaCl stress (Table 5).

In leafy vegetables, salt stress increases electrolyte leakage in lettuce and spinach leaves (Hniličková et al. 2019), inasmuch as electrolyte leakage causes cell membrane damage and electrons leak from the cell. Therefore, electrolyte leakage is reported as one of the most important criteria to determine the cell injury. However, there is no specific previous study that shows the effect of NaCl on electrolyte leakage of tomato (*Solanum lycopersicum* L. cv. Micro-Tom), and our result is the first report.

Table 5. Effect of salinity on electrons leakage in leaves and survival rate of tomato plants.

Treatments	Electrolyte leakage (%)		Survival rate (%)
	Flowering stage	Fruit-bearing stage	
Control	14.88 ± 2.00 d	17.49 ± 2.79 d	100 a
50 mM	24.03 ± 3.08 c	34.16 ± 1.80 c	100 a
100 mM	27.74 ± 3.49 c	40.47 ± 2.07 b	80 b
150 mM	33.49 ± 1.81 b	44.65 ± 1.89 a	80 b
200 mM	42.76 ± 2.11 a	47.42 ± 1.59 a	60 c
	***	***	*

Data are shown as ‘mean ± SD’, and the letters show significant differences between treatments according to the Tukey test at 0.05 level, and the asterisks show significant differences at ***p<0.001, *p<0.05.

A strong negative correlation ($r = -0.84$) was found between electrolyte leakage and number of flowers, as well as with the number of fruits ($r = -0.91$) (Fig. 4). The reason is the cell membrane injury that caused flowers to die and decrease the number of flowers, therefore, less number of fruits were formed.

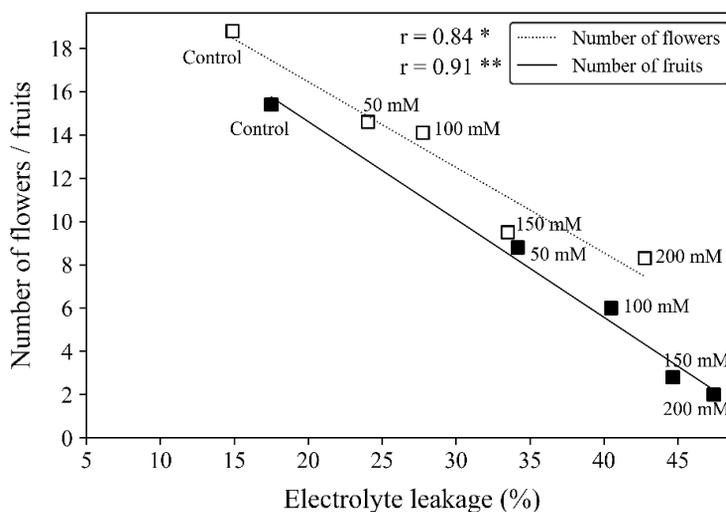


Fig. 4. The correlation coefficient of electrolyte leakage (%) with number of flowers per plant, and number of fruits per plant.

Sugar content. The most important quality indicator of tomato fruit is the presence of sugars (glucose, fructose, and sucrose). In the current study, low salinity (50 mM) improved the glucose content of tomato fruits, but high salinity level (>50 mM) gradually decreased the glucose content of tomato fruits. Furthermore, 50 mM and 100 mM NaCl improved fructose concentration, but in high levels of salinity

(150 mM and 200 mM) fructose decreased significantly as compared to control. Sucrose was highly affected by salinity, and there was a significant decrease in fruit sucrose content in salinity treatments compared to control. Therefore, salinity decreased sugar levels in tomato fruits (Table 6).

Moderate salinity (50 mM) can improve tomato fruit Brix level (Johkan et al. 2014) which is consistent with our results. A significant decrease in fruit Brix under high salinity (150 mM) has also been observed. It was also observed that 150 mM and 200 mM treatments decrease sucrose (Lu et al. 2010), and 60 mM NaCl salinity increased sugars in tomato fruits (Carvajal et al. 2000), which are consistent with results of this study.

Table 6. Effect of NaCl stress on the sugar content of tomato fruits.

Treatments	Sugars (mg/g)			
	Glucose	Fructose	Sucrose	Total
Control	42.73 ± 3.22 b	72.47 ± 3.09 c	16.65 ± 1.02 a	131.86 ± 3.97 b
50 mM	54.85 ± 3.76 a	85.08 ± 2.77 a	14.25 ± 0.76 b	154.18 ± 5.13 a
100 mM	42.04 ± 4.43 b	77.43 ± 5.60 b	12.64 ± 0.92 bc	132.11 ± 9.62 b
150 mM	34.14 ± 3.57 c	63.26 ± 1.71 d	12.12 ± 1.14 cd	109.51 ± 4.57 c
200 mM	25.01 ± 2.52 d	51.61 ± 3.12 e	10.47 ± 0.61 d	87.09 ± 5.33 d
	***	***	**	***

Data are shown as ‘mean ± SD’, the letters show significant differences between treatments according to the Tukey test at 0.05 level, and the asterisks show significant differences at ***p<0.001, **p<0.01.

In addition, we observed an improvement in fruit Brix under low and medium salinity conditions was observed, but it was significantly decreased under 150 mM and 200 mM treatments compared to control. Furthermore, there was a negative relationship between fruit Brix and fruit fresh weight under low (T1) and medium (T2) salinity, but in treatments 150 mM and 200 mM, where tomato plants face high salt stress, both fruit fresh weight and fruit Brix decreased significantly (Fig. 5).

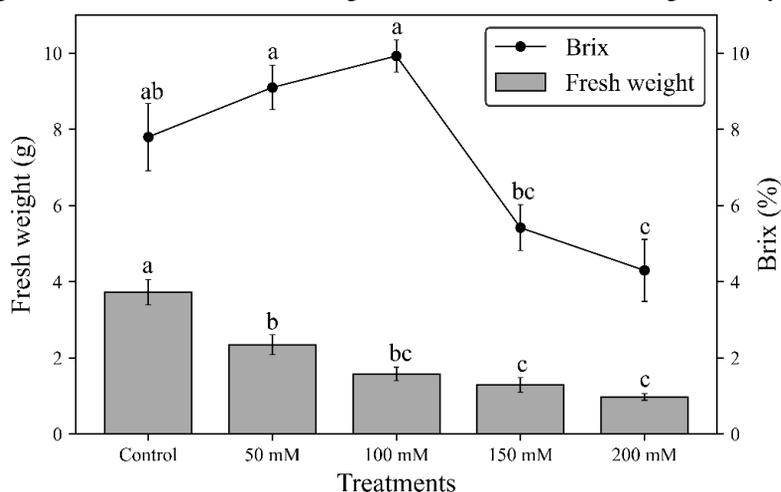


Fig. 5. Effect of salinity on fresh weight and Brix of tomato fruits. The letters represent significant differences between treatments according to the Tukey test at 0.05 level.

Moreover, salinity delayed the fruit ripening in tomato fruits (Table 8), and it is obvious that the red and ripened fruits have a higher amount of sugars. Therefore, the fruits in control plants were

fully ripened and red, but in salinity treatments, the fruits were not fully ripened, and their color was ranged from pale red (50 mM, 100 mM, and 150 mM) to green (200 mM) (Fig. 6).

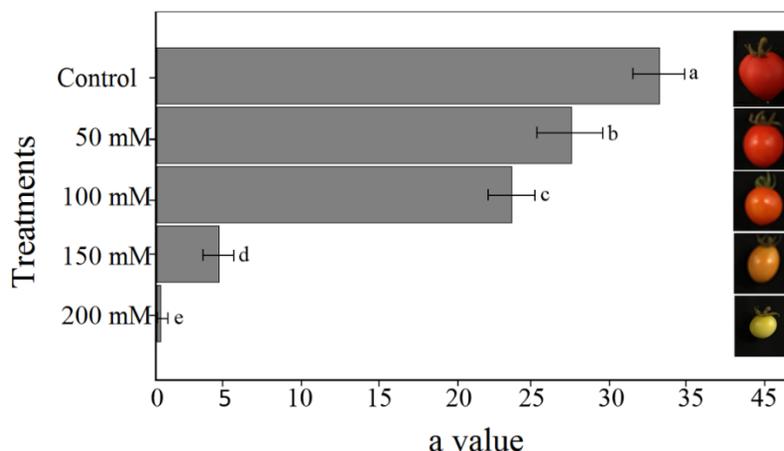


Fig. 6. The effect of salinity on tomato fruit color at ripening stage. The letters represent significant differences between treatments according to the Tukey test at 0.05 level.

Organic acid content. Organic acid was measured from the harvested fruits of the same age based on time of anthesis. There was a significant increase in organic acid accumulation in tomato fruits under NaCl stress conditions compared to control. There was a significant increase in citric acid and malic acid concentration compared with control (Fig. 7), this result agree with previous studies by Zushi and Matsuzoe (2006).

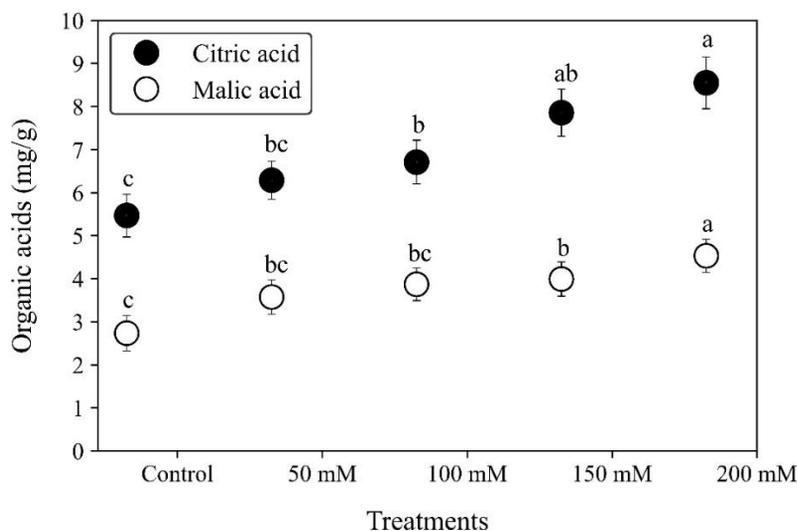


Fig. 7. Effect of salinity on organic acid content of tomato fruits. The letters represent significant differences between treatments according to the Tukey test at 0.05 level.

Malondialdehyde and proline content. Malondialdehyde (MDA) and proline are highly reactive chemical compounds produced to strengthen the plant to combat abiotic stress (Gharsallah et al. 2016). In the current study, MDA and proline concentration in tomato leaves which were grown under saline conditions produced significantly higher concentrations compared to control (Table 7). Plants grown under high salinity produced a higher concentration of proline, and the leaf size (leaf area) was smaller than control (Table 7).

Table 7. Malondialdehyde and proline content in tomato leaves.

Treatments (T)	MDA (10^{-5} nmol/g FW)	Proline (10^{-1} mg/g FW)
Control	4.92 ± 1.29 d	3.79 ± 0.31 d
50 mM	8.48 ± 0.61 c	4.16 ± 0.30 cd
100 mM	10.74 ± 2.46 c	4.99 ± 0.28 c
150 mM	14.75 ± 2.40 b	5.56 ± 0.12 b
200 mM	22.00 ± 2.23 a	6.27 ± 0.19 a
	***	**

Data are shown as ‘mean ± SD’, the letters show significant differences between treatments according to the Tukey test at 0.05 level, and the asterisks show significant differences at ***p<0.001, **p<0.01.

Ethylene production and fruit maturation. Fruit ripening has been delayed in the plants grown under saline conditions. There was a significant difference in the number of days to full fruit ripening between plants grown under control and salinity treatments. A higher number of days to fruit ripening was observed in the salinity treatments compared to control. The plants grown under control conditions took lesser days to mature their fruit compared to the ones in the salinity treatments. Furthermore, fruits produced by plants grown under the saline conditions with the same flowering date were green in color (Fig. 6) and had harder pericarp than in control (Table 8).

Ethylene production was also affected by salinity in tomato fruits. This is because of not fully ripened and unhealthy fruits in salinity treatments. There was a significant difference in ethylene production between control and saline treatments. The fruits which were grown under control had the highest ethylene production while the lowest ethylene production was measured from the plants grown under saline conditions (Table 8). Salinity induces ethylene production in plants (Zapata et al. 2017; Riyazuddin et al. 2020), but it was observed that when the control fruits were fully ripened, the salinity stressed ones were hard and not mature, therefore, their firmness was higher and ethylene production was lower compared to control ones. However, we measured ethylene production of salinity stressed plants’ fruit when they became mature and red, we observed an increase in ethylene production compared to the previous time, because this time the salinity treatments fruits were ripened (Table 8).

Table 8. Effects of salt-stress conditions on fruit ripening characteristics of tomato.

Treatments (T)	Fruit firmness (N)	Ethylene production (nl/g/hr)		Days to ripening
		Control plants maturity	Stressed plants maturity	
Control	2.99 ± 0.48 d	0.81 ± 0.26 a	0.81 ± 0.26 c	92.4 ± 2.70 e
50 mM	3.86 ± 0.69 cd	0.51 ± 0.15 b	0.85 ± 0.09 bc	97.8 ± 1.30 d
100 mM	4.22 ± 0.52 c	0.38 ± 0.11 bc	0.89 ± 0.06 b	102.0 ± 3.43 c
150 mM	5.64 ± 0.86 b	0.21 ± 0.08 c	0.91 ± 0.12 b	109.6 ± 1.81 b
200 mM	7.80 ± 0.52 a	0.09 ± 0.06 d	0.94 ± 0.10 a	113.8 ± 2.49 a
	***	**	**	***

Data are shown as ‘mean ± SD’, the letters show significant differences between treatments according to the Tukey test at 0.05 level, and the asterisks show significant differences at ***p<0.001, **p<0.01.

CONCLUSION

Salinity stress retards tomato plant growth and has adverse consequences on physiological traits such as photosynthetic rate, SPAD value, transpiration rate, stomatal conductance, and leaf temperature. Furthermore, salinity decreases the water absorption in tomato roots which leads to low transpiration rate in leaves, and it increases the leaf temperature and electrolyte leakage. Therefore, a smaller number of plants can survive under high salinity. Moreover, sodium chloride salinity stress increases fruit firmness and decreases ethylene production, and due to the presence of sodium in fruit cells that helps to produce thicker cell membrane, therefore, this characteristic delays fruit maturity.

Tomato plants are very sensitive to salinity in the reproductive stage causing fewer fruits with higher levels of acids and low sugar content. In the future we would like to find some practical and easy approaches to enhance the tomato plant tolerability against salinity.

ACKNOWLEDGMENT

We would like to acknowledge the financial support from Japan International Cooperation Agency (JICA) for the conduct of this study.

REFERENCES CITED

- Anon. 1974. Proposal for study of color spaces and color-difference evaluation. (Technical Note) J. Opt. Soc. Am. 64: 896-897
- Agius, C., von Tucher, S., Poppenberger, B., and Rozhon, W. 2018. Quantification of sugars and organic acids in tomato fruits. *MethodsX*, 5: 537–550. <https://doi.org/10.1016/j.mex.2018.05.014>
- Bam, R. K., Kumaga, F. K., Ofori, K., and Asiedu, E. A. 2006. Germination, vigour and dehydrogenase activity of naturally aged rice (*Oryza sativa* L.) seeds soaked in potassium and phosphorus salts. *Asian Journal of Plant Sciences*, 5(6), 948–955. <https://doi.org/10.3923/ajps.2006.948.955>
- Carvajal, M., Cerd, A., and Mart, V. 2000. Modification of the response of saline stressed tomato plants by the correction of cation disorders. *Plant growth regulation* 30: 37–47.
- Fetouh, M. and Hassan, F. 2014. Seed germination criteria and seedling characteristics of *Magnolia grandiflora* L. trees after cold stratification treatments. *Int. J. Curr. Microbiol. App. Sci.* , 3(3), 235–241.
- Gharsallah, C., Fakhfakh, H., Grubb, D., and Gorsane, F. 2016. Effect of salt stress on ion concentration, proline content, antioxidant enzyme activities and gene expression in tomato cultivars. *AoB PLANTS*, 8. <https://doi.org/10.1093/aobpla/plw055>
- Giannakoula, A. E., and Ilias, I. F. 2013. The effect of water stress and salinity on growth and physiology of tomato (*Lycopersicon esculentum* Mill). *Archives of Biological Sciences*, 65(2), 611–620. <https://doi.org/10.2298/ABS1302611G>
- Hatsugai, N. and F. Katagiri. 2018. Quantification of plant cell death by electrolyte leakage assay. *Bio-Protocol*, 8(5), 1–7. <https://doi.org/10.21769/bioprotoc.2758>
- Hernández-Pérez, O. I., Valdez-Aguilar, L. A., Alia-Tejacal, I., Cartmill, A.D., and Cartmill, D.L. 2020. Tomato fruit yield, quality, and nutrient status in response to potassium: calcium balance and electrical conductivity in the nutrient solution. *Journal of Soil Science and Plant Nutrition*, 20(2),

484–492. <https://doi.org/10.1007/s42729-019-00133-9>

- Hniličková, H., Hnilička, F., Orsák, M., and Hejnák, V. 2019. Effect of salt stress on growth, electrolyte leakage, Na⁺ and K⁺ content in selected plant species. *Plant, Soil and Environment*, 65(2), 90–96. <https://doi.org/10.17221/620/2018-PSE>
- Ismail Razi, M., Burrage, S. W., Ismail, M. R., and Burrage, S. W. 1994. Effects of salinity on growth, water relations and photosynthetic rate of tomatoes grown in nutrient film technique. *Pertanika J. Trop. Agric. Sci*, 17(2), 73–79.
- Johkan, M., Nagatsuka, A., Yoshitomi, A., Nakagawa, T., Maruo, T., Tsukagoshi, S., Hohjo, M. A., Lu, N., Nakaminami, A., Tsuchiya, K., and Y. Shinohara. 2014. Effect of moderate salinity stress on the sugar concentration and fruit yield in single-truss, high-density tomato production system. *Journal of the Japanese Society for Horticultural Science*, 83(3), 229–234. <https://doi.org/10.2503/jjshs1.CH-096>
- Kandil, AA., Sharief AE., and Nassar ESE.,.2012. Response of some rice (*Oryza Sativa* L.) cultivars to germination under salinity stress. *International Journal Of Agriculture Sciences*, 4(6), 272–277. <https://doi.org/10.9735/0975-3710.4.6.272-277>
- Kuehni, R. G. 1976. Color-tolerance data and the tentative CIE 1976 L*a*b* formula. *J. Opt. Soc. Am.* 66:497-500.
- Locato, V., Cimini, S., and Gara, L. De. 2013. Strategies to increase vitamin C in plants : from plant defense perspective to food biofortification. 4 (May), 1–13. <https://doi.org/10.3389/fpls.2013.00152>
- Lu, S., Li, T., and Jiang, J. 2010. Effects of salinity on sucrose metabolism during tomato fruit development. *African Journal of Biotechnology*. 9(6), 842–849. <https://doi.org/10.5897/AJB09.1602>
- Masuthi, D., Chavan, M., Rubina, K., Waseem, M., Kulkarni, S., Bhanuje, T., and Haleshkumar, B. 2015. Different priming treatments on germination and viability of cluster bean seeds. *International Journal of Advanced Research*, 3(5), 108–111.
- Parvin, K., Ahamed, K. U., Islam, M. M., and Haque, N. 2015. Response of tomato plant under salt stress: Role of exogenous calcium. *Journal of Plant Sciences*, 10(6), 222–233. <https://doi.org/10.3923/jps.2015.222.233>
- Riyazuddin, R., Verma, R., Singh, K., Nisha, N., Keisham, M., Bhati, K. K., Kim, S. T., and Gupta, R. 2020. Ethylene: A master regulator of salinity stress tolerance in plants. *Biomolecules*, 10(6), 1–22. <https://doi.org/10.3390/biom10060959>
- Saito, T., Ariizumi, T., Okabe, Y., Asamizu, E., Hiwasa-Tanase, K., Fukuda, N., Mizoguchi, T., Yamazaki, Y., Aoki, K., and Ezura, H. 2011. TOMATOMA: A novel tomato mutant database distributing micro-tom mutant collections. *Plant and Cell Physiology*, 52(2), 283–296. <https://doi.org/10.1093/pcp/pcr004>
- Singh, J., Sastry, E. V. D., and Singh, V. 2012. Effect of salinity on tomato (*Lycopersicon esculentum* Mill.) during seed germination stage. *Physiology and Molecular Biology of Plants*, 18(1), 45–50. <https://doi.org/10.1007/s12298-011-0097-z>

- Ullah, N., Basit, A., Ahmad, I., Ullah, I., Shah, S. T., Mohamed, H. I., and Javed, S. 2020. Mitigation the adverse effect of salinity stress on the performance of the tomato crop by exogenous application of chitosan. *Bulletin of the National Research Centre*, 44(1). <https://doi.org/10.1186/s42269-020-00435-4>
- Umar, J., Aliyu, A., Shehu, K., and Abubakar, L. 2018. A study on effects of salinity on growth and yield of tomato genotype (*Solanum lycopersicum*). 3, 16–24. <https://doi.org/10.18052/www.scipress.com/SFP.3.16>
- Wang, Sheng, Y., Tian, S. P., and Xu, Y. 2005. Effects of high oxygen concentration on pro- and anti-oxidant enzymes in peach fruits during postharvest periods. *Food Chemistry*, 91(1), 99–104. <https://doi.org/10.1016/j.foodchem.2004.05.053>
- Wang, W., Vinocur, B., and Altman, A. 2003. Plant responses to drought, salinity and extreme temperatures: Towards genetic engineering for stress tolerance. *Planta*, 218(1), 1–14. <https://doi.org/10.1007/s00425-003-1105-5>
- Yang, X., Li, Y., Chen, H., Huang, J., Zhang, Y., Qi, M., Liu, Y., and Li, T. 2020. Photosynthetic response mechanism of soil salinity-induced cross-tolerance to subsequent drought stress in tomato plants. *Plants*, 9(3). <https://doi.org/10.3390/plants9030363>
- Zapata, P. J., Serrano, M., García-Legaz, M. F., Pretel, M. T., and Botella, M. A. 2017. Short term effect of salt shock on ethylene and polyamines depends on plant salt sensitivity. *Frontiers in Plant Science*, 8(May), 1–13. <https://doi.org/10.3389/fpls.2017.00855>
- Zhang, P., Senge, M., and Dai, Y. 2017. Effects of salinity stress at different growth stages on tomato growth, yield, and water-use efficiency. *Communications in soil science and plant analysis*, 48(6), 624–634. <https://doi.org/10.1080/00103624.2016.1269803>
- Zhu, G., Wang, S., Huang, Z., Zhang, S., Liao, Q., Zhang, C., Lin, T., Qin, M., Peng, M., Yang, C., Cao, X., Han, X., Wang, X., van der Knaap, E., Zhang, Z., Cui, X., Klee, H., Fernie, A. R., Luo, J., and Huang, S. 2018. Rewiring of the fruit metabolome in tomato breeding. *Cell*, 172(1–2), 249–261.e12. <https://doi.org/10.1016/j.cell.2017.12.019>
- Zushi, K., and Matsuzoe, N. 2006. Postharvest glutamic acid and changes antioxidant under in sugar , organic acid, fruit contents salinity stress in tomato grown Faculty of Environmental and Symbiotic Sciences, Prefectural University of Kumamoto , *Environ. Control Biol.*, 44(2), 111–117.