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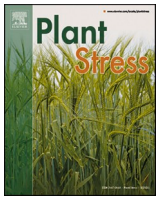

$NaHCO_3$ impairs the growth and fruit yield of tomato plants

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ABSTRACT

Underground water enriched in $NaHCO_3$ is used in farms of the Buenos Aires province for tomato crop irrigation. This farming practice leads to salt accumulation and soil impairment after several seasons of cultivation inside the greenhouses. This work assayed the effect of $NaHCO_3$ on tomato fruit production. Plants of the Elpida variety of *Solanum lycopersicum* L were grown in a hydroponic system. The presence of $NaHCO_3$ (from 5 mM, as measured in the underground water to 10 or 20 mM) reduced K^+/Na^+ ratio and whole plant biomass and fruit yield; however, no effect was observed on fruit quality parameters. To test the participation of ascorbic acid in the tolerance to this stress, two *slggp1* Micro-Tom mutant lines deficient in this antioxidant were used. In these experiments plants were treated with 0, 5 and 10 mM $NaHCO_3$ causing an impairment of K^+/Na^+ ratio, photosynthesis, fruit yield, leaf and shoot dry weight (but without effect in root biomass) and delaying of fruit ripening time. Wild type and mutants plant responses showed no differences at stress conditions. Although $NaHCO_3$ treatments caused a similar impairment in ascorbic acid mutants and wild type plants, these results reinforced the physiological importance of ascorbic acid levels to optimize plant growth under non-stressful conditions. Taken as a whole, the results presented here demonstrated the importance of avoiding the accumulation of this salt in greenhouse soils to optimize tomato production.

1. Introduction

Contaminated underground water with a variety of salts is allocated for irrigation in cultivation of different plant crops. The ions dissolved in the water may accumulate in the soils of arid and semi-arid areas or inside greenhouses after several seasons without removing the greenhouse cover. The increase of soil salinization due to irrigation has a great impact in lands dedicated to agriculture (Munns, 2002). Among these salts, the presence of $NaHCO_3$ in underground water frequently occurs in different continents. In the horticultural areas of Buenos Aires Province (Argentina) this salt constitutes a major component of the water used for irrigation (Andreau et al., 2012). The accumulation of $NaHCO_3$ deteriorates soil properties through increased compaction, decreasing permeability to water and gases. It also causes an increase in pH which reduces the availability or absorption of many mineral nutrients causing several physiological modifications in several plant species. Treatments with $NaHCO_3$ decrease growth, chlorophyll content and K^+/Na^+ ratio in shoot and root tissues in lettuce plants (Bie et al., 2004). In kiwifruit this kind of stress also causes anatomical modifications such as thickening of palisade tissue, decreased the length, width and aperture of stomata and degeneration of chloroplasts (Wang et al., 2019). This alkali stress increases the secretion of organic acids which constitutes a crucial mechanism to regulate the pH outside the roots (Yang et al., 2010). The treatments with $NaHCO_3$ (alone or in combination with CO_3^{2-}) cause an imbalance of nutrient ions and growth in tomato seedlings associated with a decrease in photosynthesis (CO_2 uptake), stomatal conductance and increase of oxidative stress (Wang et al., 2015; Gong et al., 2013). However, the effects of this salt in the whole growth cycle and fruit yield

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in tomato have been scarcely addressed. This research is aimed at studying these traits in tomato plants and the participation of some physiological mechanisms behind them.

It is interesting that salinity treatments have been successfully used to improve some characteristics of tomato fruit quality such as total soluble solids (Saito et al., 2008; Yin et al. 2010). However, the irrigation with sodic water (containing CO_3^{2-} + HCO_3^-) caused a decrease in tomato yield but did not improve fruit quality (Choudary et al., 2010). This study also showed that ascorbic acid concentration, an index of fruit quality, increased in a salt tolerant fruit irrigated with the sodic water. Ascorbic acid constitutes a crucial antioxidant in plant tissues with many functions in the protection of photosynthetic metabolism (Foyer and Noctor, 2011).

This work tests the hypothesis that $NaHCO_3$ may affect plant growth and fruit yield through changes in tissues ion accumulation, ascorbate content and photosynthesis. The concentrations of ions were analyzed in roots, leaves and fruit, ascorbate contribution to salt tolerance was assayed with Micro-Tom Knock-Out mutant plants for the GDP-L-galactose phosphorylase (GGP) which is described as the most controlling enzyme of the ascorbic acid synthesis pathway. In addition, photosynthesis was determined through gas exchange and chlorophyll fluorescence analyses.

2. Material and Methods

2.1. Plant material and treatments

Experiments were carried out with plants of *Solanum lycopersicum* L at the Institute of Plant Physiology (National University of La Plata), Argentina (34°91′ S, 57° 93′ W). The first trials were carried out with *Solanum lycopersicum* L var Elpida (cultivated by growers in local farms) plants. Additional experiments were performed with wild type *Solanum lycopersicum* L cv Micro-Tom plants and two ethyl methanesulfonate (EMS) mutant plants deficient in the ascorbic acid content. These two mutant lines, named GGP-5261 and GGP-P49C12, were generated at the University of Tsukuba (Japan) and INRA Bordeaux (France), respectively. The tomato genome contains two GGP genes, namely GGP1 and GGP2. The GGP mutants used here corresponded to two independent Knock-Out mutations of the most expressed GGP protein in tomato, namely GGP1, which resulted in the reduction by 80 to 90% of the ascorbic acid content (Baldet et al., 2013; Just et al., 2013). *Solanum lycopersicum* L var Elpida plants were cultivated in a greenhouse at 700 μmol photons m^{-2} s^{-1} at midday and temperatures of 25 ± 2 and 20 ± 3 ºC during the day and night, respectively, in spring and summer. Micro-Tom plants were grown in a chamber at 400 μmol photons m^{-2} s^{-1} at a 16/8 h light/dark photoperiod and 26 ± 3 ºC. Hydroponic systems were used to grow plants in all experiments as described in Fanello et al. (2020). The seeds were sown in trays with a nutrient enriched (N: 200; P: 15; K: 200 ppm) soil mixture of peat and perlite (1:1) and transferred to the hydroponic system after two weeks of germination. Treatments consisted in the addition of 0 to 20 mM $NaHCO_3$ to the hydroponic solution as indicated for each experiment. The pH of hydroponic solution increased from 5.8 to about 7.6 (from 0 to 20 mM $NaHCO_3$, respectively).

2.2. Soil analysis

Prior to the experiments, soils from horticultural establishments that exhibited salinity-sodicity symptoms were analyzed. To do this, composite samples were taken from the top 20 cm of the tomato cultivation ridge. The determinations of Na^+ and electric conductivity (EC) were performed in saturated extract paste according to Rhoades et al. (1999) and Merani et al. (2020). The concentration of Na^+ in the soil was determined by using flame photometry while EC was determined by conductimetry (SAMLA-PROMAR, 2004).

2.3. Plant biomass, fruit quality and ripening time determinations

Dry weight was measured in fruit, leaves, shoots and roots after dehydration in an oven at 68 ºC until weight no longer changed. The fruit quality parameters, pH, titratable acidity (TTA) and total soluble solids (TSS) were measured as in Steelheart et al. (2019). Samples were composed by combining 10 g aliquots from different fruit and processed with mortar and pestle. The pH of the juice was potentiometrically determined with a pHmeter (Hanna Edge®) and TTA was measured with a 0.1 mol L^{-1} NaOH solution until reaching a pH of 8.2 (AOAC, 1980). TTA was expressed in g of citric acid per kg of fruit fresh weight. In order to determine the TSS content, tomato juice was placed in a refractometer (Milwaukee MA871, Rocky Mount, USA) and expressed as percentage.

Ripening time was determined as the time from mature green to red ripe stages of the fruit still present on the truss (Steelheart et al., 2020). Fruit set was calculated as the percentage of flowers that developed into fruit (flower number/fruit number x100).

2.4. Ascorbic acid measurements

Ascorbic acid concentration was measured in fruit, leaves and roots by a HPLC system (Shimatzu LC-10Atvp solvent delivery module and UV-Vis SPD-10Avp detector) according to Bartoli et al. (2006) and Aguilera et al. (2020).

2.5. Ion concentration measurements

One g of dried tissue powder of fruit, leaves and roots was heated at 500ºC in a muffle for 4 to 8 h and then heated to boil in 2 M HCl. After cooling and filtered through filter paper the solution is brought to the final volume with water and used for ion determinations. Na^+, K^+ and Ca^{2+} were measured by atomic-absorption spectrophotometry (Perkin Elmer, PinAAcle 900F) as described by Sadzawka et al. (2007).

2.6. Photosynthesis measurements

An infrared gas analyzer (PLC 6, Cirus-2 PPSystems) was used for quantification of CO_2 uptake (A_{max}) and stomatal conductance. The photosynthetic electron transport rate (ETR), Fv/Fm and non-photochemical quenching (NPQ) were measured with a modulated chlorophyll fluorescence system (FMS-2, Hansatech Instruments Ltd., Norfolk, UK, Genty et al., 1989). Photosynthesis was measured at saturated irradiance (1200 μmol photons m^{-2} s^{-1}) inside the growth chamber. Fv/Fm was determined after dark adaptation of leaves for 30 min.

2.7. Statistical analysis

Data were obtained from at least three independent experiments and expressed as the means ± standard error (S.D.). Statistical analysis was conducted using the InfoStat® 2020 software package (https://infostat.com.ar/). For continuous quantitative variables (v.g. individual fruit weight) an ANOVA analysis and post hoc tukey tests were performed ($P<0.05$). Two types of analyses between treatments on fruit number and fruit setting, a generalized linear model (GLM), were used: a Poisson family and a log link function for number of fruit and binomial family and a logit link function for fruit setting ($P<0.05$) and post hoc LSD tests were performed ($P<0.05$).

3. Results

3.1. Effects of $NaHCO_3$ treatments on tomato cv Elpida plants

In a preliminary survey, we observed that the soils in farms dedicated for the cultivation of tomatoes around the city of La Plata (Buenos Aires province, Argentina), contain Na^+ in concentrations varying from 0.7 to

around 60 meq L^{-1} or eventually higher, with an EC of 0.3 to 13 dS m^{-1} and sodium absorption ratio (SAR) of 0.9 to 10. The lowest values correspond to soils in open fields whereas the highest ones correspond to soils inside greenhouses after some years without removing the cover. This salt accumulation is caused by the irrigation with underground water (where we measured an EC of 7.0 to 7.8 dS m^{-1}) classified as sodium bicarbonate by Andreau et al. (2012). To assay the effect of this salt on tomato crops, plants were treated with 0 to 20 mM $NaHCO_3$ included in the solution of a hydroponic system.

The first experiments studied the yield of a tomato variety usually cultivated by growers under different concentrations of $NaHCO_3$. Although the lower concentrations (similar concentrations to that observed in underground water) had no effect, higher levels of $NaHCO_3$ in the hydroponic solution decreased fruit yield per plant (Fig. 1A). Either the reduction in the number of fruits per plant (Fig. 1B) or in the individual fruit weight (Fig 1C) were involved in the lower fruit biomass. Lower fruit numbers were associated with reduced set efficiency under stress conditions (Fig. 1D). In addition, other experiments showed that vegetative plant biomass, leaf area and the chlorophyll content decreased under salt treatment (Supplementary Fig. 1A-C) but the water consumption by the whole plant was not affected (Supplementary Fig. 1D).

The $NaHCO_3$ treatments increased the concentrations of Na^+ in fruit, leaves and roots of tomato plants; however, only leaves showed a decreased concentration of K^+ (Table 1) under $NaHCO_3$ treatments.

Fruit ripening parameters like pH, TSS, TTA and TSS/TTA ratio were not affected by $NaHCO_3$ treatments (Table 2).

Table 1
Effect of $NaHCO_3$ treatments on Na^+ and K^+ concentrations in fruit, leaves and roots of *Solanum lycopersicum* L var Elpida plants.

		Na^+	K^+	K^+/Na^+
Fruit	0 mM	0.015± 0.003a	2.9 ±0.23a	205.0
	5 mM	0.066 ±0.05b	3.12 ±0.37a	49.6
	15 mM	0.12 ±0.014c	3.7 ±0.011a	31.2
	20 mM	0.21 ±0.02d	2.5 ±0.18a	12.3
Leaves	0 mM	0.112 ±0.008a	3.15 ±0.2a	29.1
	5 mM	0.377 ±0.046b	2.65 ±0.12ab	7.3
	15 mM	0.51 ±0.035bc	2.35 ±0.12b	4.8
	20 mM	0.567±0.097c	2.80 ±0.1ab	6.0
Roots	0 mM	0.32 ±0.1a	2.53 ±0.4a	11.3
	5 mM	1.5 ±0.7b	2.64 ±0.23a	2.3
	15 mM	3.01 ±0.23c	2.65 ±0.37a	0.9
	20 mM	1.63±0.044b	2.2 ±0.61a	1.4

Results were obtained from three independent experiments. Values represent the means ± S.D. and letters indicate statistical differences at ANOVA, $P<0.05$.

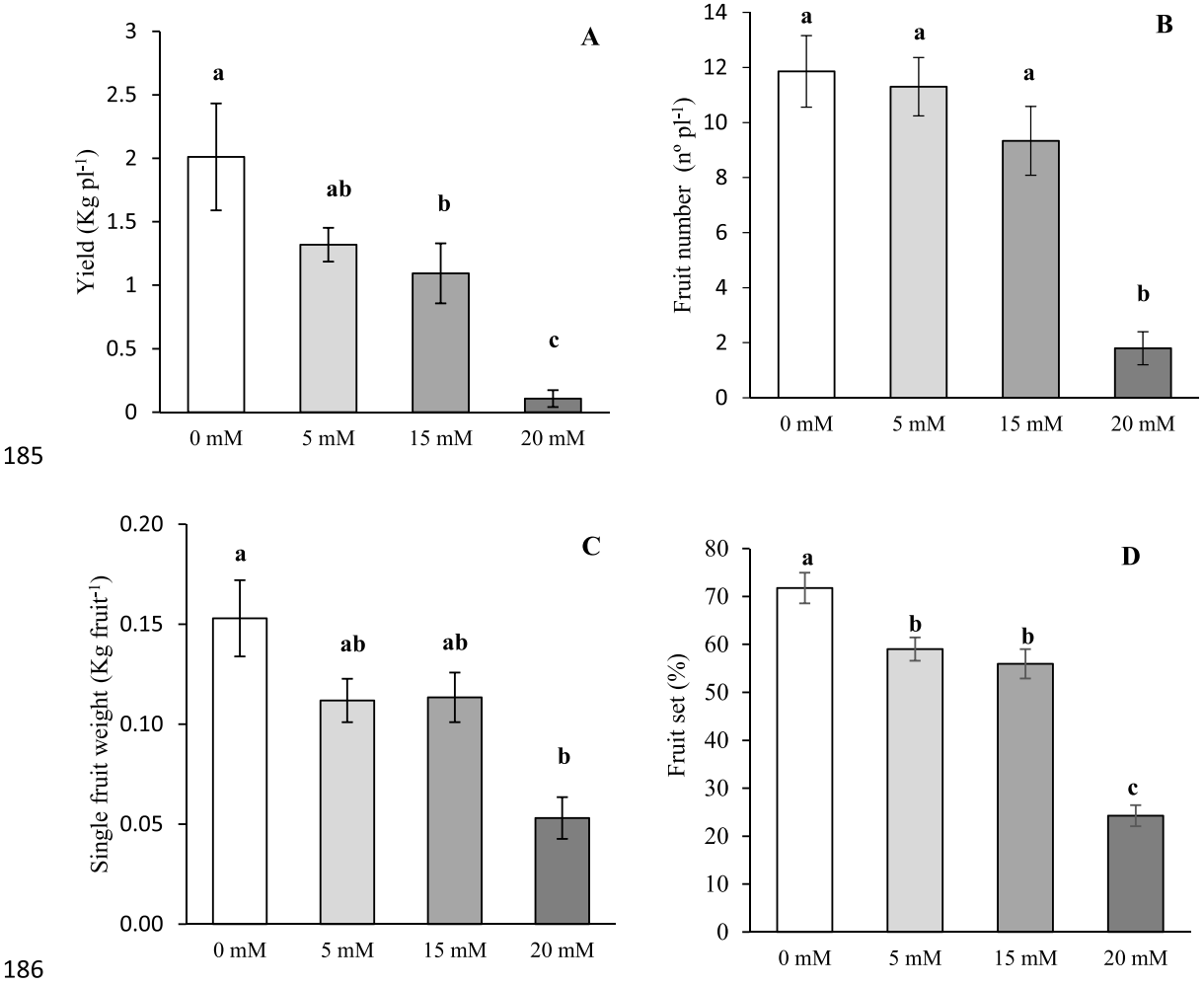

Fig. 1. Effect of $NaHCO_3$ on the yield of *Solanum lycopersicum* L var Elpida plants. A. Tomato production per plant; B. Fruit number; C. Single fruit weight and D. Fruit set. Data were obtained from three independent experiments and expressed as the means ± S.D. (ANOVA, $P<0.05$). To analyze the number of fruits per plant and fruit setting a generalized linear model was used: a Poisson family and a log link function for number of fruits, binomial family and a logit link function for fruit setting ($P<0.05$).

Table 2
Effect of $NaHCO_3$ treatments in pH, TSS (total soluble solids), TTA (total titratable acidity) and ratio (TSS/TTA) during fruit ripening of *Solanum lycopersicum* L var Elpida plants.

	pH	TSS (°Brix)	TTA (g citric acid kg^{-1} FW)	Ratio (TSS/TTA)
0 mM $NaHCO_3$	4.4 ±0.03a	5.46 ±0.41a	0.43±0.02a	12.62±0.74a
5 mM $NaHCO_3$	4.38 ±0.06a	5.21 ±0.47a	0.45±0.04a	12.61±2.13a
15 mM $NaHCO_3$	4.25 ±0.01a	5.33 ±0.22a	0.55±0.03a	9.71±0.27a
20 mM $NaHCO_3$	4.42 ±0.08a	5.37 ±0.34a	0.42±0.07a	13.64±2.98a

Results were obtained from three independent experiments. Values represent the means ± S.D. and letters indicate statistical differences at ANOVA, $P<0.05$.

3.2. *Effects of $NaHCO_3$ on ascorbic acid deficient tomato mutant plants*

Micro-Tom *Slggp1* mutant plants were used to test the participation of ascorbic acid in the tolerance against $NaHCO_3$ treatments. Since concentrations as high as 20 mM produce a large impact in plant growth and fruit set, we decided to perform these experiments in the range of 0 to 10 mM of $NaHCO_3$. The ascorbic acid content was not affected by $NaHCO_3$ treatments in fruit or leaves of any genotype (Fig. 2A-B); however, it increased in roots of the wild type plants treated with 5 and 10 mM $NaHCO_3$ (Fig. 2C). The oxidized state of ascorbate increased in fruit of all genotypes and in the wild type leaves of treated plants (Fig. 2A-B). Treated or untreated root tissues resulted with ascorbate in a highly oxidized state (Fig. 2C).

Sodium concentration increased similarly in treated plants of all genotypes and the leaves reached the highest concentrations (Table 3). Potassium contents decreased with increasing $NaHCO_3$ treatments in leaves and roots but this effect in fruit was observed only in the GGP49C12 mutant. An accumulation of calcium was observed in roots of all genotypes at the highest salt concentration but was not altered in leaves and fruit. No effect was observed in iron concentration in any of the treatments (Supplementary table 1).

The whole plant fresh weight was reduced in all genotypes by $NaHCO_3$ treatments (Supplementary Fig. 2). Among vegetative organs, leaf and shoot dry weights were decreased in all genotypes at 10 mM $NaHCO_3$ but the root dry weight increased with salt treatment showing only a statistical difference in GGP49C12 plants (Fig. 3 A-C). The fruit dry weight per plant was reduced by 27.7 and 41.6 % at 5 and 10 mM taking the average for wild type and mutants (Fig. 3D). This was associated with lower fruit number in wild type and ascorbic acid deficient mutants (Fig. 3E). Additionally, salt treatments increased the ripening time of all genotypes (Fig. 3F). Photos of wild type and *slggp1* mutant plants are shown in Supplementary figure 3 grown in a hydroponic system untreated or treated with 5 and 10 mM $NaHCO_3$.

Photosynthesis was assayed through different approaches. CO_2 uptake (A_{max}) was higher in wild type plants than in mutants under either control or stress conditions and inhibited at 10 mM $NaHCO_3$ treatment in plants of all genotypes (Fig. 4A). Stomatal conductance in wild type plants were higher than in mutants and decreased at 10 mM $NaHCO_3$ (Fig. 4B); however stomatal conductance of both mutant plants was not affected by salt treatments. A negative effect was observed in ETR of wild type leaves at the highest salt treatment showing a reduction of about 13 % with no effect in any mutant (Fig. 4C). Fv/FM measured to assay the damage of PSII, showed a photosynthetic impairment at the highest salt treatment condition (Fig. 4D). Increased heat energy dissipation determined as NPQ further suggested a stressed state in plants at the 10 mM $NaHCO_3$ concentration (Fig. 4E).

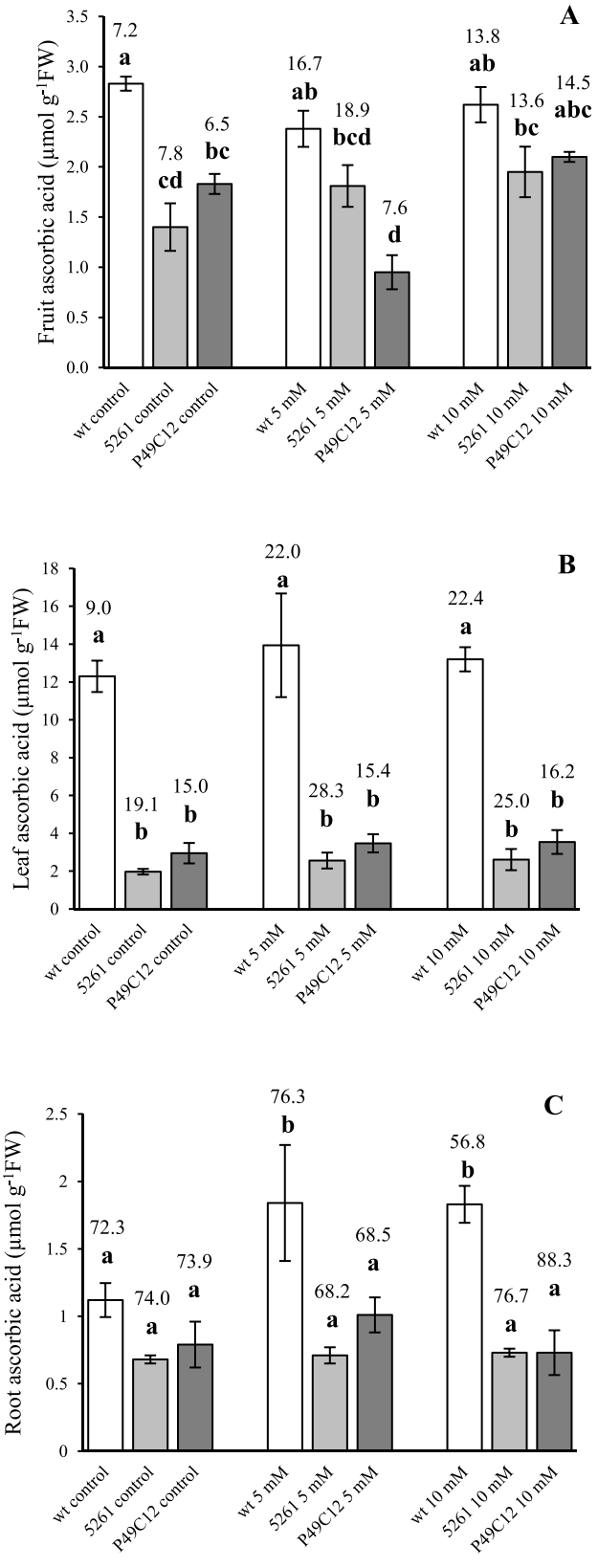

Fig. 2. Effect of $NaHCO_3$ on the total ascorbic acid content and oxidized state of wild type and ascorbic acid deficient mutant organs. A. Fruit; B. Leaves and C. Roots. Data were obtained from three independent experiments and expressed as the means ± S.D. (ANOVA, P<0.05). The oxidized ascorbic acid state is shown above of each bar and expressed in percentage of the total ascorbate.

Table 3
Effect of $NaHCO_3$ treatments on Na^+, K^+ and Ca^{2+} concentrations in wild type and ascorbic acid deficient mutant fruit, leaves and roots. Data are expressed in % (w/w).

		Na^+	K^+	K^+/Na^+	Ca^{2+}
	wt 0 mM	0.06± 0.02a	3.1±0.15ab	63	0.47 ±0.2 a
	5261 0 mM	0.04 ±0.016a	3.3 ±0.2 a	123	0.56 ±0.23 a
	P49C12 0 mM	0.049 ±0.015a	3.3 ±0.3 a	95	0.6 ±0.25 a
	wt 5 mM	0.23 ±0.02bc	2.9 ±0.18ab	13	0.54 ±0.18 a
Fruit	5261 5 mM	0.18 ±0.03b	2.8 ±0.4ab	15	0.62 ±0.22 a
	P49C12 5mM	0.206 ±0.03b	3.0 ±0.16ab	15	0.64 ±0.23 a
	wt 10 mM	0.35 ±0.1cd	2.5 ±1ab	7	0.84 ±0.33 a
	5261 10 mM	0.23 ±0.09bc	2.5 ±0.7ab	11	0.84 ±0.33 a
	P49C12 10 mM	0.38 ±0.1d	2.1 ±1.4b	5	0.89 ±0.38 a
	wt 0 mM	0.34 ±0.11ab	5.6 ±0.74 a	19	6.0 ±1.1 a
	5261 0 mM	0.31 ±0.09a	5.76 ±0.62 a	20	4.4 ±0.76 a
	P49C12 0 mM	0.21 ±0.01a	5.62 ±0.19 a	26	5.5 ±1.1 a
	wt 5 mM	0.91 ±0.13cd	4.15 ±0.13b	5	4.92 ±0.5 a
Leaves	5261 5 mM	0.77 ±0.11bc	4.18 ±0.16b	6	4.63 ±0.7 a
	P49C12 5mM	1.03 ±0.09cde	4.16 ±0.32b	4	4.51 ±0.6 a
	wt 10 mM	1.38 ±0.2def	3.67 ±0.19b	2.7	5.55 ±1.1 a
	5261 10 mM	1.46 ±0.3ef	3.65 ±0.07b	2.6	5.15 ±0.9 a
	P49C12 10 mM	1.65 ±0.68f	3.55 ±0.07b	2.4	4.94 ±0.8a
	wt 0 mM	0.227 ±0.1ab	6.72 ±0.4a	51	1.53 ±1.0a
	5261 0 mM	0.086 ±0.07a	6.53 ±0.15 a	325	1.37 ±0.85 a
	P49C12 0 mM	0.107 ±0.04ab	5.67 ±0.9ab	63	1.54 ±1.0 a
	wt 5 mM	0.387 ±0.07bcd	5.14 ±0.37abc	14	3.36 ±1.2ab
Roots	5261 5 mM	0.32 ±0.05abc	4.8 ±0.32bc	16	3.5 ±1.1ab
	P49C12 5mM	0.32 ±0.09abc	4.84 ±0.82bc	17	3.5 ±1.1ab
	wt 10 mM	0.68 ±0.3de	3.5 ±0.6c	6	5.4 ±1.6b
	5261 10 mM	0.515 ±0.13cde	3.55 ±0.07c	7	5.26 ±1.3b
	P49C12 10 mM	0.68 ±0.11e	3.7 ±0.7c	6	5.44 ±2.0b

Results were obtained from three independent experiments. Values represent the means ± S.D. and letters indicate statistical differences for the same organ at ANOVA, $P < 0.05$.

4. Discussion

Tomato cultivation in greenhouses require the continuous irrigation of plants. The use of water containing ions like Na^+ and HCO_3^{2-} leads to an increase of soil pH, Na^+ concentration and deterioration of physical properties that may impact crop production. This contamination with $NaHCO_3$ is characteristic of groundwater used in farms in the province of Buenos Aires (Argentina, Andreau et al., 2012) but the presence of this salt is also prevalent in water and soils in other cultivated areas around the world (Stavi et al., 2021). Previous works report detrimental effects of alkali stress on tomato plants at the vegetative stage (Jumberi et al., 2002; Wang et al., 2015; Gong et al., 2013) but its impact on tomato fruit yield is scarcely studied. To test the effect of this salt on tomato fruit production, we treated plants with increasing concentrations of $NaHCO_3$ using 5 mM as the lowest concentration, as it is present in underground water used for irrigation.

Treatments with $NaHCO_3$ in the hydroponic solution decreased the biomass production and the yield of tomatoes in different genotypes (i.e. Elpida and Micro-Tom, Figs. 1 and 2). Biomass reduction was previously observed in plantlets of tomato at higher $NaHCO_3$ concentrations than used in this work (Jumberi et al., 2002). Earlier works (Tanaka and Fujita, 1974; Alegre et al., 2020) state that the number of fruits is the main trait defining tomato yield. In line with these works, the lower fruit yield observed here in $NaHCO_3$ treated plants is associated with a decreased fruit set efficiency leading to a reduced fruit number (Fig. 1).

Treatments with $NaHCO_3$ affected the levels of different mineral nutrients in tomato seedlings (Gong et al., 2013). Here, all plant organs showed an increased Na^+ accumulation but leaves also showed a decreased concentration of K^+ in both Elpida and Micro-Tom cultivars. Decreases in K^+ concentrations observed in leaves may impair several processes, decreasing plant growth (Srivastava et al., 2020). Since changes in K^+/Na^+ ratio may affect leaf metabolism, we focused next on the analysis on photosynthesis. CO_2 uptake and other photosynthetic parameters are impaired by different kinds of alkaline treatments (Gong et al., 2013; Wang et al., 2015) in tomato seedlings. Although lower concentrations did not affect photosynthesis, higher $NaHCO_3$ concentration decreased CO_2 uptake and ETR (Fig. 4). Besides this, the decrease of Fv/Fm suggests that impairment in photosynthetic components is produced by $NaHCO_3$ treatments. Reduced photosynthetic activity may provoke a lower biomass accumulation and fruit production. The use of water enriched in sodium carbonates also provoked lower tomato yield (Choudary et al., 2010). Although photosynthesis likely limits biomass production, it could also alter the supply of sugars to the fruit, which can affect several ripening-associated changes (soluble solids, color, aroma, or others; Osorio et al., 2014; Durán-Soria 2020). Most fruit quality parameters were not affected by $NaHCO_3$ treatments used here, which nevertheless delayed the "in vine" ripening time. Shortening of the time to reach the red stage in fruits is usually observed following different types of abiotic stress like salinity, high irradiance and high temperature (Mizrahi et al., 1982; Steelheart et al., 2022; Ruiz-Nieves et al., 2021). This discrepancy may be associated with different kinds and strengths of stress with changes in hormones such as ethylene and abscisic acid that control the progress of ripening traits (Kou et al., 2021). Further studies must include experiments to unravel the effect of these $NaHCO_3$ treatments in hormone concentration and signaling.

Ascorbic acid participates in several physiological processes by improving the acclimation of plants to a changing environment with several functions in leaf metabolism (Paciolla et al., 2019). Its participation in the water-water cycle by removing excess reactive oxygen species and in the dissipation of excess energy load as a cofactor of violaxanthin deepoxidase critically contributes to optimize photosynthesis. *Slggp1* mutants deficient in ascorbic acid displayed hormonal changes, like decreased gibberellin concentration and higher ethylene production, lower photosynthetic activity and reduced fruit yield (Alegre et al., 2020). Liu et al. (2015) showed that melatonin treatment increases ascorbic acid, besides other antioxidants, which was associated with alleviation against physiological disorders caused by alkaline stress in tomato seedlings. In addition, exogenous ascorbic acid treatments improved plant performance under saline conditions in other species (Azeem et al., 2023; Hasanuzzaman et al., 2023). However, *Slggp1* mutants deficient in ascorbic acid showed lower CO_2 uptake (A_{max}) than wild type in either untreated or $NaHCO_3$ treated plants. This lower photosynthetic activity of both mutants at 10 mM might be caused by the lower gas exchange since ETR, Fv/Fm and NPQ were similar to the wild type. Taking together the results showed that photosynthesis was impaired by $NaHCO_3$ decreasing stomatal conductance and causing some damage in the chloroplasts. The lower photosynthesis and stomatal conductance observed in *Slggp1* mutants under control conditions

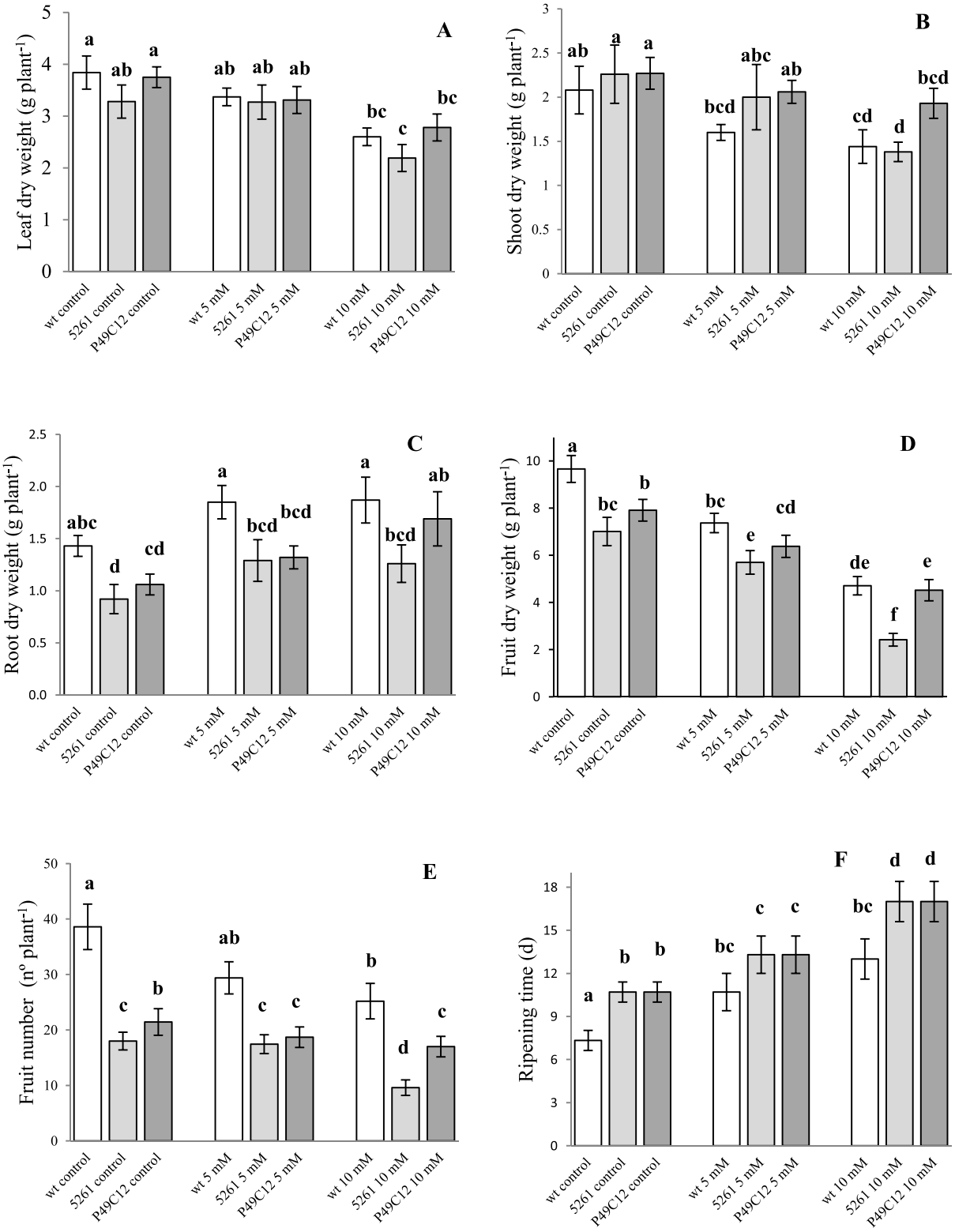

Fig. 3. Effect of $NaHCO_3$ in wild type and ascorbic acid deficient mutant plant organs. A. Leaf dry weight; B. Shoot dry weight; C. Root dry weight; D. Fruit dry weight; E. Fruit number per plant and F. Fruit ripening time to change from mature green to red stage. Data were obtained from three independent experiments and expressed as the means ± S.D. (ANOVA, $P < 0.05$). To analyze the number of fruits per plant, a generalized linear regression model was used: a Poisson family and a log link function ($P<0.05$).

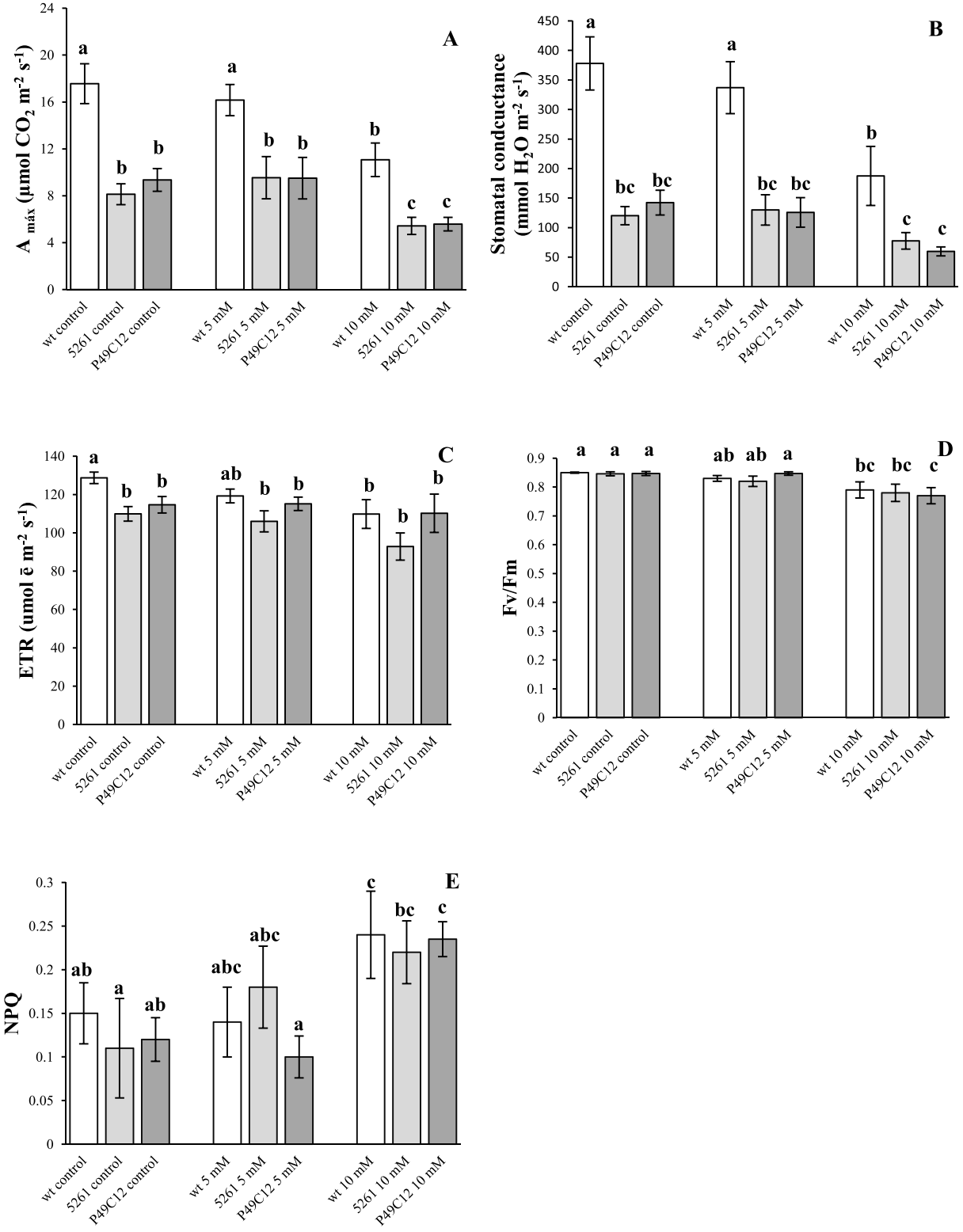

Fig. 4. Effect of $NaHCO_3$ on photosynthesis in wild type and ascorbic acid deficient mutant plants. A. CO_2 assimilation at saturating irradiance; B. Stomatal conductance; C. ETR; D. Fv/Fm and E. NPQ. Data were obtained from three independent experiments and expressed as the means ± S.D. (ANOVA, $P < 0.05$).

which was kept under $NaHCO_3$ treatments suggests that the deficiency of this antioxidant proned the plants to a "stress behavior" even at a non-stressful environment. The restriction to plant performance induced by ascorbic acid deficiency under optimum conditions confirmed the crucial role of this antioxidant for plant growth (Plumb et al., 2018).

Finally, it is noteworthy that while the $NaHCO_3$ treatments affected the growth of fruit and leaves, the biomass of the roots of Micro-Tom plants that were directly exposed to this salt did not change. This

result showed an organ-dependent sensitivity to this mild abiotic stress.

5. Conclusion

The concentration of $NaHCO_3$ present in the water used for irrigation causes a moderate impairment in tomato plants; however, higher concentrations lead to a severe impact like decreased yield, leaf biomass, photosynthesis or delay of fruit ripening time. These results suggest that accumulation of this salt must be avoided to keep higher tomato crop production under greenhouse conditions. We are currently performing field experiments in commercial farms to associate changes in environmental factors, such as soil pH or nutrient concentration changes, with tomato yield. In addition, we are studying some physiological mechanisms like the participation of organic acids (specifically malate metabolism), nitric oxide and plant hormones in the tolerance to alkali stress.

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CRediT authorship contribution statement

Inti M. Ganganelli: Investigation. **Matías L. Alegre:** Investigation, Supervision. **Charlotte Steelheart:** Investigation. **Pierre Baldet:** Investigation, Writing – original draft. **Christophe Rothan:** Writing – review & editing. **Cecile Bres:** Investigation. **Daniel Just:** Investigation. **Yoshihiro Okabe:** Investigation. **Hiroshi Ezura:** Writing – review & editing. **José Vera Bahima:** Investigation. **Guillermo Millán:** Investigation. **Gustavo E. Gergoff Grozeff:** Investigation. **Carlos G. Bartoli:** Investigation, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2024.100354.

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