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IMPACT OF SALINE-ALKALI STRESS ON THE ACCUMULATION OF SOLIDS IN TOMATO FRUITS

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Abstract

Growing of tomato plants in saline conditions, having high rhizospheric EC, is often reported with high solid content in fruits. However, saline-alkali stress conditions, having high rhizospheric pH as well as high EC, have never been studied to evaluate its impact on the solid content of tomato fruits. In this study, we investigated the impact of saline-alkali stress (0, 30, 60, 90, and 120 mM $NaHCO_3$) on the accumulation of solids in tomato fruits. Addition of sodium bicarbonate ($NaHCO_3$) to plants highly increased pH as well as EC of the soil leachate in 90 and 120 mM $NaHCO_3$ treatments in comparison to control treatment. Saline-alkali stress treatments did not influence the fruit dry weight, nonetheless, the content of fruit dry matter was increased significantly from 6.5% at control to 8.5% at 90 and 120 mM treatments. The content of soluble sugar was increased to 3% in 90 mM treatment in comparison to control (2%), owing to significant accumulation of hexose as well as sucrose in ripe fruits. In addition to carbohydrates, saline-alkali stress influenced the accumulation of organic acids in fruits, as well. Citric acid, being the major acid, showed positive correlation with the salt concentration, and was significantly high at stress treatments of higher than 30 mM. These results suggested that saline-alkali stress conditions, in spite of high pH, can increase the contents of fruit solids in tomato, as is usually observed in saline stress conditions.

Introduction

In tomato, fruit solids comprise less than 10% of the fresh weight (Davies & Hobson, 1981), therefore, an increase in solid content is very important for quality improvement. High solid content of fruits may enhance consumers' demand and market value of the fresh produce as well, besides increasing its processing efficiency (Stark *et al*., 1996). In tomato fruits, soluble sugar and organic acids, being the major soluble solids, contribute about 70% to the total solid content of fruits (Bertin *et al*., 2000; Guichard *et al*., 2001), thereby influence the taste and flavour of the fruits (Salunkhe *et al*., 1974; Ho, 2003).

Solid content of fruits is governed by certain genetic and environmental factors. Among environmental factors, exposing plants to saline or water deficit stress conditions are usually reported with improved fruit solid content (Mizrahi *et al*., 1988; Mitchell *et al*., 1991a, 1991b; Renquist & Reid, 2001; Patane & Cosentino, 2010), particularly with high sugar and organic acids contents (Ho *et al*., 1987; Gao *et al*., 1998; Sakamoto *et al*., 1999; Plaut *et al*., 2004; Sato *et al*., 2006; Saito *et al*., 2008). Likewise, manipulation of leaf-fruit ratio can alter the sucrose concentration of phloem sap entering into fruits and thereby the solid content of fruits (Jan & Kawabata, 2011).

Soil salinity is a serious problem for plant growth in most countries of arid to semi arid regions in the world. In such soils, because of high dissolved salts (mostly NaCl), plant growth and fruit yield may decrease (Ali *et al*., 2011; Azeem & Ahmad, 2011; Saqib *et al*., 2012), but solid content of fruits is reported to increase which may compensate for low yield. Literature regarding improvement of solid content in tomato fruits under stress conditions is mostly focused on NaCl or water deficit stresses. Both of these stress conditions may increase electrical conductivity (EC) of the nutrient solution in the root biosphere or lower water potential of the soil without affecting pH of the growing medium.

However, those soils which contain $NaHCO_3$/$NaCO_3$ as dominant salts are characterized by high EC as well as high pH. Those soils, owing to high EC and pH, may have different stress conditions from saline stress; because plants would have to withstand both elevated EC as well as pH in the rooting zone (Yang *et al*., 2007; 2008a, 2008b). Such soils are termed as saline-alkali soils and are even larger in area than saline soils in the world. Out of the total arable land in the world (7×10^9 ha) only 21.5% (1.5×10^9 ha) is under cultivation (Tanji, 1996). Saline-alkalinity affects 37% of the cultivated land, whereas salinity affects 22.5%, and both these soils are spread in more than 100 countries of the world (Tanji, 1996). Saline stress is known to increase fruit solid content, especially in tomato (Ho *et al*., 1987; Mitchells *et al*., 1991a; Yin *et al*., 2010), however, it is not clear whether saline-alkali stress (high EC and pH) can also influence fruit solid content. Saline-alkali and saline stresses are regarded two different stress conditions (Yang *et al*., 2007; 2008a, 2008b), but the influence of saline-alkali stress on fruit solid content has scarcely been studied, either in hydroponic culture or soil medium, in tomato crop. In this experiment, we tried to evaluate the impact of saline-alkali stress on the accumulation of solids in tomato fruits.

Materials and Methods

Plant materials: Tomato seeds (*Solanum lycopersicum* L. cv. 'House Momotaro') were obtained from Takii Seed (Kyoto, Japan). During summer 2009, seeds were soaked for 24 hours on a blotting paper and subsequently sowed in soil compost in a glasshouse. After germination seedlings were watered with tap water. With the unfolding of the 4th leaf, plants were transferred to 5-L pots, containing peat-based soil mixture (Soil Mix, Sakata Seed, Yokohama, Japan) and granulated soil (Engei Baido, Kureha, Tokyo, Japan) in equal proportion.

All the plants were grown under sunlight in a glasshouse having proper ventilation to avoid high temperature. Plants were trained to a single stem through regular pinching of axillary buds. Fruit were set on the first truss of the plant and the upper part of the plant was severed at fruit set to maintain a proper source-sink balance. Upon anthesis, first flower of the truss was pinched off and the subsequent two flowers were sprayed with a synthetic plant-growth-regulator (Tomato tone, Ishihara, Japan) for uniform fruit setting.

Saline-alkali treatment: After transplanting, all the plants were watered for one week with tap water and fertilised with half-strength Otsuka nutrient solution (Osaka, Japan). After one week, plants were subjected to four saline-alkali treatments (30, 60, 90, and 120 mM) or kept untreated as the control.

For saline-alkali treatments, sodium bicarbonate salt ($NaHCO_3$), dissolved in tap water at concentration of 30, 60, 90, and 120 mM, was applied to the plants. Each plant was given 1L sodium bicarbonate (salt) solution twice a week at 2 to 3 days interval. These plants were fertilised with 1L half strength Otsuka nutrient solution twice a week, usually one day prior to the salt treatments. Control plants were fertilised, twice a week, with 1L half-strength Otsuka nutrient solution and irrigated, two-time weekly, with 1L tap water.

Soil leachate pH/EC determination and fruit sampling: In all treatments, after applying salt or nutrient solutions to the plants, the percolated solutions were collected form each plant until the last drop. Every week, one time each after salt and nutrient solution application, i.e., twice a week, pH and EC of the leachate were recorded. In control plants, EC and pH were recorded only in the leachate after applying nutrient solution. About 30 ml of soil leachate was sampled in 50 ml tube and their EC was measured with portable EC meter (CM-14P, TOA Electronics Ltd., Japan) and pH meter (210, Beckman Instruments, Inc., USA).

At the breaker stage, i.e. the appearance of pink colour on fruit, salt application was stopped and, when needed, plants were watered with tap water, in equal volume, just to keep the soil moisture. At full-ripe stage, 4-5 days after the breaker stage, both of the two fruits on the truss were harvested for analysis at the dusk. Each fruit was weighed and then cut vertically into four radial segments. A slice was taken from two segments on opposite direction and stored at -18ºC.

Analysis of carbohydrates and organic acids: Carbohydrates and organic acids were extracted from fruits and analysed as reported previously (Jan & Kawabata, 2011). Fruit samples were freeze-dried and ground in a mortar with pestle. About 100 mg samples were boiled in 80% ethanol, 2 hours for sugar and 30 minutes for acid extraction, at 85ºC. The extracts were filtered through Whatman GF/F filter paper (25 mm, England) and the filtrates were dried in a rotary evaporator under vacuum. The extracts were redissolved in 10 ml deionised water.

For sugar analysis, an aliquot of sample was passed through ion-exchange resin (Amberlite MB-3) column for sugar analysis. The eluates were centrifuged at 15,000 rpm, 4ºC for 10 minutes. An aliquot of the supernatant was diluted twice with water and subjected to 10A-HPLC (Shimadzu, Kyoto, Japan) equipped with RI-101 refractive index detector (Shodex, Tokyo, Japan). Sugars were separated through a CARBOSep CHO-620 column (6.5 mm I.D x 300 mm, Transgenomic) at 90ºC. The mobile phase was degassed Milli-Q water at a flow rate of 0.5 ml min^{-1}.

For organic acid analysis, a sample solution mixed with internal standard (succinic acid) was diluted with water and the acids were separated by TSK gel ODS 100 V column (4.6 mm I.D. x 250 mm, 5 µm, Tosoh, Japan) at 40ºC on Shimadzu 10-A HPLC system equipped with SPD-10AV UV-VIS detector (Shimadzu, Kyoto, Japan) set at 210 nm. Phosphoric acid (0.1 %) was used as mobile phase with a flow rate of 0.8 ml·min^{-1}.

For starch extraction, the ethanol-insoluble fraction was boiled in 10 ml water at 100ºC for 2 hours. The liberated starch was hydrolysed through amyloglucosidase (EC 3.2.1.3, 142 U mg^{-1}, 10113, Sigma) in 1 ml of 0.2 M Na-acetate buffer (pH 4.5) at 37ºC over night. The glucose contents were determined enzymatically using Glucose assay Kit, (Glucose CII, Wako) and starch content was estimated.

For the experiment, plants were arranged according to randomised complete block design (RCBD) in the glasshouse; each treatment having 10 replications. Data were analysed according to one-way analysis of variance (ANOVA) and means were compared by using Student-Newman-Keuls test in case of significant result.

Results

Soil leachate pH and EC: Mean soil leachate EC was 2.3 dS m^{-1} in control treatment whereas, addition of sodium bicarbonate salt enhanced EC of soil leachate up to 5.5 dS m^{-1} in 120 mM treatment (Fig. 1A). Likewise, soil leachate pH was 6.5 in control treatment, however, pH in soil leachate was above 8 in 90 and 120 mM treatments (Fig. 1B).

Fruit size and its total solids: Tomato fruit fresh weight (FW) was decreased only in 90 and 120 mM saline-alkali treatment, whereas, fruit size, estimated from cross sectional area, remained unaffected in comparison to control (Fig. 2A, B). Saline-alkali treatments (30-120 mM) had no significant influence on the accumulation of fruit dry weight (DW) in comparison to the control treatment (Fig. 2C). However, unlike DW, the content of fruit dry matter (DM), on a fresh weight basis, was significantly increased at above 30 mM treatment. Minimum DM was observed in control plants (6.8%), while a maximum of 8.5% was recorded in 90 and 120 mM treatments (Fig. 2D).

Soluble sugar and organic acids accumulation: Glucose and fructose, being the major accumulated sugars in ripe fruits, were accumulated in almost equal proportion by all treatments. Glucose concentration was 0.9% in the control, which increased gradually with salt concentration reaching up to 1.4% at 90 mM treatment. Likewise the lowest content of fructose was in control (1%), whereas the highest (1.4%) was in 90 mM treatment (Fig. 3E, F). In ripe fruits, the content of sucrose was much more lower (0.07-0.2%) than hexoses, but saline-alkali stress significantly increased their content in higher than 60 mM treatments (Fig. 3G). Starch content of the fruit was non-significant in all treatments except 120 mM (Fig. 3H). Total soluble sugar increased significantly to 3% in 90 mM treatment as compared to control i.e., 2% (Fig. 3I).

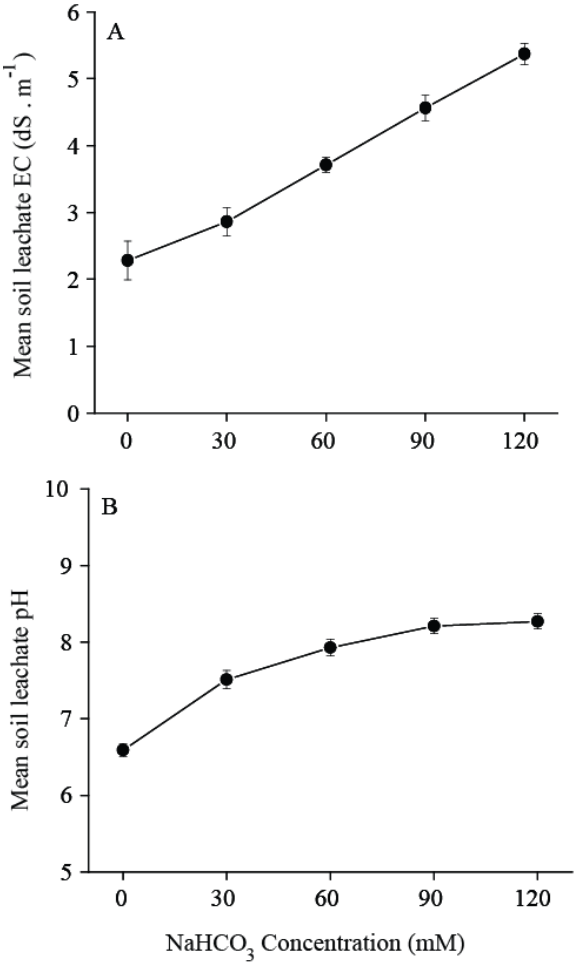

Fig. 1. Effect of various concentrations of Sodium bicarbonate salt (30, 60, 90 and 120 mM $NaHCO_3$) and control treatments (0 mM) on the pH and EC of soil leachate. A, B show mean data regarding EC and pH of the soil leachate. Error bars indicate mean ± SE. Each point represents mean value of n = 7-10.

Citric acid was the major accumulated organic acid in the ripe fruit, followed by malic and maleic acids. The content of citric acid was significantly higher in stress treatment particularly at 90 and 120 mM treatments (25 and 32 $mg \cdot g^{-1}$ FW respectively) in comparison to control treatments (19 $mg \cdot g^{-1}$ FW), however, the contents of malic and maleic acids were not affected by stress treatments (Fig. 3J-L).

Discussion

Fruit fresh and dry weight accumulation: In this study the influence of saline-alkali stress on soluble solid accumulation in tomato fruit, at the ripe red stage, was evaluated. Sodium bicarbonate salt, when added to the soil, increased the soil pH as well as EC, as is reflected in the soil leachate data (Fig. 1A, B). Such high pH, could be due to bicarbonate ions in the pot soil. Saline-alkali stress reduced fruit size and fresh weight at high treatments. Fruit enlargement is suggested to be regulated by the transport of water and assimilated-carbon into the fruit (Ho, 1996), similarly, reduction in tomato fruit FW, owing to high root-zone EC, is attributed to decrease in fruit radius and cell size because of low water influx (Saito *et al.*, 2006, 2009). Therefore, it can be assumed that low fruit FW and size in 90 and 120 mM treatments could be because of reduction in water influx only, as dry weight accumulation of fruits was unaffected (Fig. 2C).

Fruit dry weight was not affected significantly within the whole range (0-120 mM) of saline-alkali stress treatments. Nonetheless, fruit dry matter on fresh weight basis improved in 60 mM treatment by 20% and in the higher saline-alkali treatments by 25% (Fig. 2C, D). High fruit solids in stress conditions, as compared to control, could be due to metabolic alteration in fruits (Balibrea *et al*., 1996). These results also indicate that saline-alkali stress may improve fruit solid content, as is usually reported in saline conditions (Ho *et al.,* 1987; Mizrahi *et al*., 1988; Mitchell *et al*., 1991a, 1991b; Yin *et al*., 2010; Arshad *et al*., 2012).

Sugar and organic acids accumulation: Glucose and fructose were the major accumulated sugars and their accumulation was regulated in similar pattern under the saline-alkali stress treatments (Fig. 3E, F). Their concentration was increased by 50% in 90 mM treatments as compared to control. Apart from hexose, sucrose concentration was also increased, (140% and 200% in 90 and 120 mM treatments, respectively), instead of its very low content in ripe fruit. Starch analysis at ripe stage of fruit revealed very low content, probably due to its breakdown into hexoses (Robinson *et al*., 1988). Likewise, total soluble sugar in fruits of 90 mM treatment was 55% higher than control, while in the other salt treatments total soluble sugar was higher but the variance was non significant as compared to control. The increase in fruit soluble sugar, under stress conditions (90 mM), could be due to the combined effect of i) preferential allocation of imported carbon to fruit (Saito *et al*., 2009), and therein to starch reserve (Gao *et al.,* 1998), resulting in high starch accumulation in early developmental stage of fruits (Balibrea *et al*., 1996; Yin *et al*., 2010), and ii) prolonging the period of starch synthesis in fruit (Gao *et al.,* 1998), whose hydrolysis subsequently may add to the hexose pool (Dinar & Stevens, 1981). Accumulation of sucrose in 90 and 120 mM stress-treatments was increased on fruit dry weight basis as well (data not shown), therefore, condensation of fruit constituents may not be the sole reason in this work. Some intricate changes, like preferential allocation of imported assimilates into sugars or some other pathways of sugar synthesis inside fruit could be involved. Saito *et al*., (2008) has suggested the involvement of gluconeogenesis pathway in the accumulation of sugars in tomato fruits under saline-stress conditions. Likewise, considering the role of high rhizospheric pH in accumulation of sugar in fruits would not be high speculation as McEnroe and Coulter (1964) has reported an increase in sugar content and sugar yield of sugar beet crop when soil pH increased from less than 6.0 to above 7.0.

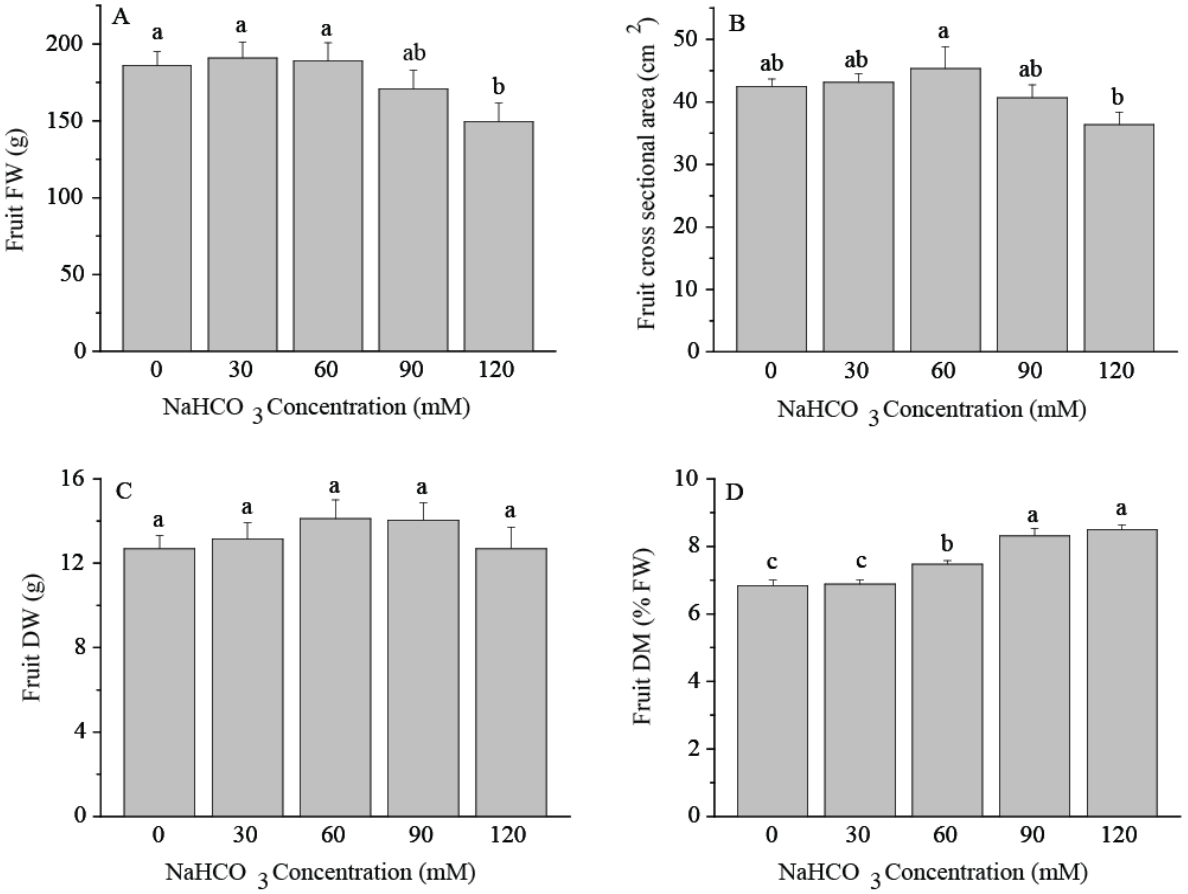

Fig. 2. Effect of saline-alkali stress (0-120 mM $NaHCO_3$) on fruit fresh weight (A), cross sectional area (B), dry weight (C), and percent dry matter (D) in tomato fruits grown under glasshouse in soil compost. Error bars indicate means ± SE. The number of replicates are n = 7-10. Different letters over the bars show significant difference among treatments ($p<0.01$) as determined by ANOVA and separated by Student-Newman-Keul's Multiple Range Test.

Apart from sugars, organic acids are the second major component of fruit soluble solids and influence fruits flavour as well. Citric and malic acids are the major organic acids in ripe tomato fruits in most cultivars (Suarez *et al*., 2008). They account for about 15% of the fruit solid content (Davies & Hobson, 1981). The content of fruit malic and maleic acids were non-significant in all treatments however, citric acid content was significantly high in saline-alkali stressed plants (Fig. 3J-L). The content of citric acid in fruits was increased by 25 - 80% as salt concentration increased from 60 to 120 mM L^{-1} in comparison to control plants. Citric acid showed a positive correlation (r = 0.965) with the concentration of sodium bicarbonate salt. Excessive accumulation of citric acids appears a strategy of plants, that are exposed to stress, to cope with the unfavourable environment (Guo *et al*., 2010), which may arise from the excessive uptake of Na^+ and HCO_3^- ions in roots. Under saline conditions, as the concentration of Na^+ ions is high in root zone, therefore the content of Na^+ can enhance in tomato fruit due to increased uptake from medium (Mitchell *et al*., 1991a). Moreover, in high salt conditions, K^+ is reported to serve as the major cation in fruit for cellular osmotic adjustment (Ho *et al*., 1987; Mitchell *et al*., 1991a; Bolarin *et al*., 2001; Plaut *et al*., 2004). Therefore, high accumulation of alkali ions (Na^+, K^+) coupled with low accumulation of anion (Cl^-, SO_4^{2-}) possibly change the cation/anion ratio (Mitchell *et al*., 1991a) and/or cytosolic (~7.5) and vacuolar pH (~ 5.5) (Taiz & Zeiger, 2006). There are numerous reports underpinning the accumulation of organic acids in tomato fruits as a strategy of the plant to balance the accumulated cations in fruit cells (Davies, 1964; Mitchell *et al*., 1991a), and to keep cellular metabolism undisturbed. As in plant tissues, organic acids generally exist in ionised form, as anions (Sweetman, *et al*., 2009), it appears that tomato fruit may synthesise and/or accumulate organic acids as a strategy not only to balance the ionic ratio but also buffer the vacuolar pH (~5.5) as a mechanism of cellular homeostasis. This might be the reason that the accumulation of citric acids is kept escalating in response to increase in the sodium carbonate stress levels unlike the accumulation of total soluble sugars in fruits (Fig. 3I, L).

In conclusion, this study indicated that saline-alkali stress, having high rhizospheric EC as well as pH, can increase solid content, total soluble sugar, and organic acids in tomato fruits, as is usually reported in high rhizospheric EC treatments (saline stress). Since saline-alkali stress conditions have high rhizospheric pH as well, therefore, it would be very interesting to understand whether high rhizospheric pH, alone or in association with EC, play a role in solid accumulation in tomato fruits.

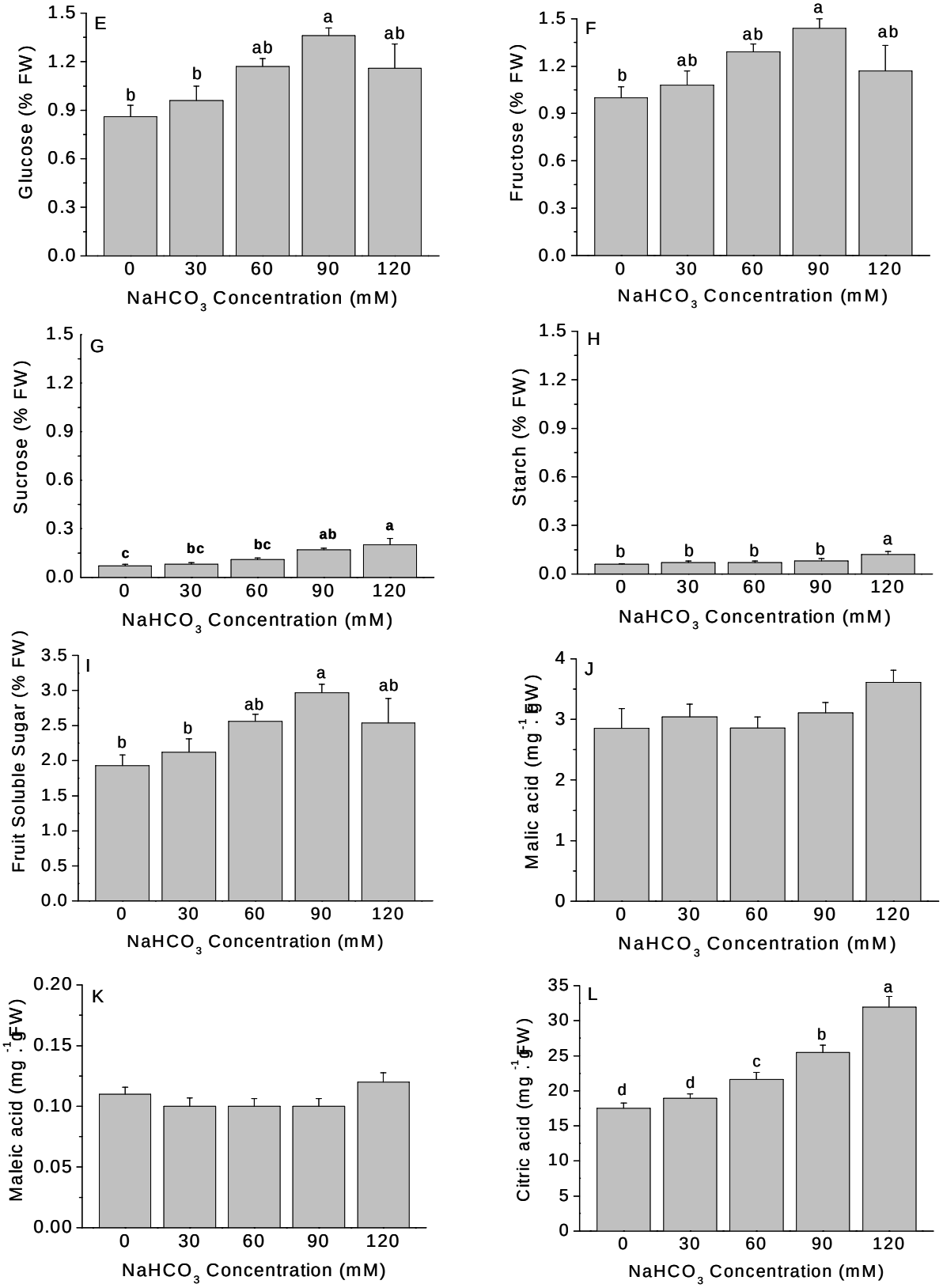

Fig. 3. Effect of saline-alkali stress (0-120 mM $NaHCO_3$) on the content of glucose (E), fructose (F), sucrose (G), starch (H), total soluble sugar (I), malic acid (J), maleic acid (K) and citric acid (L) in tomato fruits grown under glasshouse in soil compost. Error bars indicate means ± SE. The number of replicates are n = 7-10. Different letters over the bars show significant difference among treatments ($p<0.01$) as determined by ANOVA and separated by Student-Newman-Keul's Multiple Range Test.

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