Physiological studies on the regulation of solid accumulation in tomato fruits

（トマト果実における乾物蓄積の調節に関する生理学的研究）

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In the name of Allah, the Beneficent, the Merciful.

(慈悲あまねく慈愛深きアッラーの御名において。)

To My Beloved Parents

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All glory and praise be to Allah, without His special blessing I should have never been able to reach this important milestone in life. I am very thankful to Allah, The Glorified and the Exalted, Who blessed me with the quest for knowledge, the potential to pursue it up to this level, and the determination to keep the seeking of knowledge continue for ever.

Our Prophet, the Messenger of Allah (peace be upon him), has told us “One who does not thank people does not give thanks to Allah, either.” (Tirmidhi, 1955; Abu Dawud, 4811).

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N. E. Jan

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Acronyms

EDTA	Ethylenediaminetetraacetic acid
HPTS	8-hydroxypyrene-1, 3, 6- trisulfonic acid trisodium salt
UV	Ultraviolet
PPFD	Photosynthetic photon flux density
LFR	Leaf to fruit ratio
FW	Fresh weight
DW	Dry weight
FS	Fruit size
DM	Dry matter
SC	Solid content
TSS	Total soluble sugar
OA	Organic acids
SE	Standard error
EC	Electrical conductivity
LSD	Least significant difference
FAO	Food and Agriculture Organisation
MIC	Ministry of Internal Affairs and Communications

Chapter − 1

General Introduction

Since its domestication, centuries back in the present day Latin America, tomato has emerged an important horticultural crop for human consumption throughout the world. Probably because of its nutritional importance and health benefits, the cultivation of tomato has been spread, from tropics to temperate climate, worldwide. After its domestication in Mexico, tomato was introduced into Europe in 16th century, therefrom, in 18th century it arrived in Japan (Costa and Heuvelink, 2005).

Tomato is one of the most consumed vegetables and, due to its commercial importance as a fresh/processed commodity, is cultivated intensively around the world. Latest available global data show that in 2008, about 5.7 million metric tons of tomato worth $13.5 billion (Int. $) was produced in the top 20 tomato producing countries (FAOSTAT, 2009). In Japan tomato is cultivated on an area of about 13000 ha, with total production of 0.75 million tons (MIC Statistics Bureau, 2010), amounting to $173 million (FAOSTAT, 2009).

As consumers prefer fruit with high sugar and organic acid content (Sato et al., 2006), tomato growers in Japan, and the rest of developed world, focus on produce of high solid content. Apart from the fresh market demand, tomato with high solid content may have high processing efficiency as well. Stark et al. (1996) reported that the removal of water from tomato, ‘the concentration step’, to produce processed products inflict high cost in the processing industry. Therefore, they suggested high solid content of fruit a principal index for its processing efficiency. Accordingly, it appears very important to understand the physiological as well as molecular basis of accumulation of fruit solid.

The regulation of solid accumulation in tomato has been studied for a long time but

the present available literature does not seem enough to fully elucidate this crucial topic. It has been reported in various horticultural crops that the accumulation of solid in fruits is regulated by the developing fruits themselves, i.e. sink-dependently (Marcelis, 1992; Heuvelink, 1997; Valantin et al., 1999). However, other studies which investigated different tomato genotypes for the fruit solid content (Hewitt and Stevens, 1981) or worked on enriched ambient CO_2 concentration for tomato (Islam et al., 1996) reported high soluble solids in fruits. These observations suggest that the accumulation of soluble solids may be regulated source-dependently. In tomato, phloem is suggested the principal pathway for water transport into fruits (Ho et al., 1987; Araki et al., 2004; Plaut et al., 2004; Guichard et al., 2005) along with the sucrose synthesised in leaves. Therefore, changing the leaf to fruit ratio (LFR) may alter the concentration of sucrose in the phloem sap and thereby the solid content in fruits. Although some work have estimated the content of organic matter in phloem sap entering into fruits and the fruit solids in tomato plants grown under saline stress conditions (Ho et al., 1987; Plaut et al., 2004), but the relationship among LFR, sucrose concentration of the phloem sap, and fruit solid content has never been reported.

Apart from balancing source-sink ratio, the exposure of tomato plants to saline/water deficit stress conditions is suggested to enhance the solid content of fruits (Mizrahi et al., 1988; Mitchell et al., 1991a, b; Sakamoto et al., 1999; Sato et al., 2006). Salinity has become a serious soil problem which affects plant growth and yield in every continent. Under such conditions, on one hand salinity adversely affects plant growth and the overall yield (Mitchells et al., 1991b), but on the other hand it increase the solid content/quality of the produce (Sato et al., 2006). By now enough data is available to conclude that salinity decrease fruit yield but increase fruit solid content, particularly in tomato.

Apart from salinity, the world soil also has another serious problem where excess of dissolved salts ($NaHCO_3$/$NaCO_3$) affect both EC and pH of the soil solution. Such affected soil, termed sodic or saline-alkali, is far larger in area than saline soil. Out of the total cultivated land in the world (1.5×10^9 ha), 37% is affected by saline-alkalinity and 23% by salinity (Tanji, 1996). Under saline-alkali stress condition, plants have to face high rhizospheric pH in addition to elevated EC as a result of dissolved salts. Moreover, in saline-alkali and saline conditions, the cations and anions of the dissolved salts could be very different and, probably, so their effect on plants as well. Some recent reports have focused on this issue and they suggest saline-alkali stress and saline stress to be two distinct stresses (Yang et al., 2007, 2008a, b, 2009; Liu et al., 2010). These reports also reveal that plants have very different response with respect to solid accumulation under these two stress conditions.

Most of the studies on saline-alkali conditions have been conducted on non-horticultural crops, and what will be the effect of such conditions on solid accumulation in tomato fruit has not been investigated so far. Understanding the influence of saline-alkali stress on the solid accumulation of fruits can help us improve fruit quality as well as broaden the existing knowledge for the genetic improvement of new varieties to exploit such stress conditions as well.

This study was conducted to understand the relationship between the solid content of fruits and the concentration of phloem sucrose under different source-sink ratios and to understand the impact of saline-alkali stress on the solid accumulation in fruit and the possible role of soil pH in the response.

Chapter-2

2. Impact of phloem sucrose concentration on the soluble solids of tomato fruits at various source-sink ratios

2.1 Introduction

Solid content of fruit not only defines its nutritional value but also gives an insight into the allocation of carbon-compound to these sink organs. Like all soft fruit, water is the major component of tomato fruit (over 90%), while the remaining portion is solid (Davies and Hobson, 1981; Plaut et al., 2004). Of the fruit's total solids about 2/3 are soluble sugars and organic acids (Bertin et al., 2000; Guichard et al., 2001), which can influence the overall taste and flavour of the fruit (Salunkhe et al., 1974). This proportion of solids may be manipulated either genetically or through environmental factors (Hewitt and Stevens, 1981; Ho et al., 1987; Marcelis, 1993a, b, c; Islam et al., 1996; Heuvelink, 1997; Bertin et al., 2000; Lechaudel et al., 2005; Petreikov et al., 2009).

There has been a long argument about whether dry matter accumulation in fruit is controlled source-dependently or sink-dependently. When the number of fruits on a plant was increased, the dry weight yield of fruits increased proportionally to the number of fruits in tomato (Heuvelink, 1997), cucumber (Marcelis, 1992) and melon (Valantin et al., 1999), as long as the fruit load was below the threshold level. When the fruit load was above the threshold, increasing fruit load also affected individual fruit growth and then total dry weight started to saturate (Marcelis, 1993b; Heuvelink, 1997). These reports suggested that fruit dry matter accumulation is regulated sink-dependently unless fruit load is extremely high.

However, other reports suggested that soluble solids content of fruits is regulated source-dependently. Hewitt and Stevens (1981) compared tomato genotypes differing in percentage fruit solid content and observed that the genotype with higher solid contents had high leaf area per fruit throughout fruit development. In addition, environmental

conditions, such as high irradiance level on leaves in cucumber enhanced percent dry matter (Marcelis, 1993b), and high leaf ambient CO_2 level in tomato, increased the hexose concentrations of the fruits (Islam et al., 1996). These observations suggest that, unlike total fruit dry weight (DW), fruit soluble solids and percent dry matter content is determined by source leaf photoassimilation.

In tomato, the contribution of the phloem and xylem flow to the total influx of water in fruit was estimated and the results indicated that phloem conduit was the prime route for water translocation (Ho et al., 1987; Plaut et al., 2004; Guichard et al., 2005). Low proportion of water influx via the xylem may be due to the presence of hydraulic resistance to xylem flow at the knuckle of the pedicel (Lee, 1989; Van Ieperen et al., 2003; Rancic et al., 2010) or within the fruit pericarp (Malone and Andrews, 2001). Since sucrose synthesised in leaves are transported via the phloem, fluctuation in phloem sap sucrose can, therefore, influence the ratio of solids to water delivered to the sink fruits. In this regard, we hypothesized that, while total fruit DW, which may be regulated by the phloem unloading process, is determined sink-dependently, percent solid content is determined source-dependently by the concentration of the phloem sap sucrose formed in the photosynthesizing leaves.

The relationship among the source-sink ratio, phloem sap sugar concentration and fruit solid content is of particular importance, but this relationship has scarcely been investigated. Probably this is because of the difficulty in the direct evaluation of phloem sap that enters the tomato fruit. In various species, phloem sap was collected from leaves through the insect stylectomy technique (Kawabe et al., 1980; Gaupels et al., 2008) and the EDTA-chelating method (King and Zeevaart, 1974; Helden et al., 1994), while in cucurbits, sap from the stem was collected by the cut exudation method (Richardson et al., 1982; Walz et al., 2004). From the cut end of tomato pedicel, Araki et al. (1997) reported phloem

sap collection by the EDTA-chelating method, but the net phloem and xylem volumes were not differentiated. However, these studies were not focused on the relationship of the phloem sap sugar to fruit quality.

In this study, we quantified the sucrose concentration of the phloem sap collected from fruit pedicel by a modified EDTA-chelating method, and thereby evaluated the relationship among fruit dry weight, solid content of fruits, and the sucrose concentration of the phloem sap, in plants with a broad range of LFRs.

2.2 Materials and Methods

2.2.1 Plant materials

Seeds of tomato (*Solanum lycopersicum* L.) 'House Momotaro' were obtained from Takii (Kyoto, Japan). Plants were grown in 5-L pots containing 1 : 1 mixture of peat-based soil (Soil Mix, Sakata, Japan) and granulated soil (Engei Baido, Kureha, Japan) in a glasshouse controlled at above 15ºC at night. Twenty to 30 plants were sown at 3- to 4-weeks intervals in spring and autumn of 2008 and 2009. Fertilization was performed with half-strength Otsuka nutrient solution (Otsuka Chemical Co. Ltd., Japan) twice a week. Plants were watered with tap water when needed. Each time, enough water/nutrient solution was given until it leached out of the pots. Plants were arranged according to randomized complete block design and trained to a single stem through regular pinching of axillary buds. Tomato plants usually produce 9 leaves preceding the first truss, and then every succeeding truss is formed at an interval of 3 leaves. The first truss of the plant was removed at its anthesis. On the second truss, the first flower of the truss was pinched off and the remaining fruits were set by applying synthetic plant hormone, 'tomato tone' (Ishihara Co. Ltd, Japan) uniformly. Sap was collected from the second fruit of the truss.

2.2.2 Treatments

At anthesis of the second truss, plant stem was heat-girdled to check cross translocation of photo-assimilates between the first and second source-sink units consisting of one truss and three leaves below the truss. For heat-girdling a piece of cotton was wrapped around the stem just above the first truss and hot water (+90ºC) was poured on with a glass funnel. Heating can make phloem sieve tubes non-functional without damaging xylem vessels (Guichard et al., 2005). Above the second truss plants were kept growing but newly emerging leaves were pinched regularly to diminish the photo-assimilate translocation from the upper part of the plant (Fig. 1). Applying these treatments the second source-sink

unit was isolate from the other part of the plants. The required leaf / fruit ratios (LFR) of 0.2, 0.4, 0.6, 0.75, 1, 1.5, 2, and 3 (corresponding to 1/5, 2/5, 3/5, 3/4, 1/1, 3/2, 2/1, and 3/1 leaves $fruit^{-1}$) were maintained for this unit through defoliation and flower removal.

For LFRs where the number of leaves was less than three on a source-sink unit (1/5, 2/5, 1/1, and 2/1), the lower leaves of the source-sink unit were removed at the fruit set of the second flower while the remaining upper leaves were kept intact as per their treatments. For all LFRs, the second flowers of the truss were allowed to set fruits. When the number of fruit per truss was more than one, fruit setting was continued until the required number from second fruit onward in succession, and the remaining flowers were pinched off. Two weeks after the fruit set of the second flower, the plants were shifted to a growth-chamber (1.8 m x 1.8 m) controlled at 23ºC and 70% relative humidity. In the growth chamber, plants were acclimatized for 4 - 5 days at 23ºC and 70% relative humidity, receiving a PPFD of 400 $\mu mol \cdot m^{-1} \cdot s^{-1}$ at the height of the second truss of the plant for 14 hours a day. Since the growth chamber could accommodate 4 plants at a time to avoid the problem of light interception by nearby plants, plants were grown in the glasshouse and shifted to the growth chamber continuously. A single plant was considered as a replicate and the number of replicates was 9 except for the treatments of LFR 1/5 and 2/5, where the number of replicates was 4.

2.2.3 Collection of phloem and xylem exudates from the pedicel

Phloem and xylem influx from the pedicel was collected via the EDTA exudation method, as reported for tomato (Araki et al., 1997), and was modified for the quantification of net phloem sap. First fruit of the truss was severed with a sharp razor. Pedicel, ventral to the knuckle, was slightly lubricated with white petroleum jelly while taking care that the cut end remained untouched. The cut end was incubated in 20 mM EDTA (pH 7.0) solution for 5 minutes (in half 1.5 ml tube), and a very thin slice was re-excised. Then, the pedicel was

washed with EDTA solution in half 1.5 ml microtubes for two hours (Fig. 2). The washing solution was replaced every 30 minutes. All these collections were discarded. Subsequently, the cut end was wiped softly with tissue paper and the sap was collected in 200 µl EDTA + HPTS solutions (20 mM EDTA + 1 mM HPTS [8-hydroxypyrene-1, 3, 6- trisulfonic acid trisodium salt]) for one hour. The collecting tube was wrapped with parafilm to avoid evaporation loss (Fig. 2). This collection contained the mixture of both phloem and xylem exudates. EDTA minimise callose formation by Ca^{2+} in phloem vessels, thereby enhances sap exudation (King and Zeevaart, 1974). HPTS is a cell membrane impermeable florescent probe and its dilution by exuded sap could help quantify the volume of net collected sap. The fruit pedicel is somewhat fuzzy and some solution that adheres to the trichomes can be lost via capillary action out of the tube. To prevent such loss petroleum jelly was applied around the pedicel.

After an hour of collection, the pedicel was re-incubated in the EDTA solution. The basal side of the abscission layer was wrapped with a piece of cotton and heat-girdled (Fig. 2). After 30 minutes, pedicel was wiped gently and xylem sap was collected for an hour in fresh 200 µl EDTA+HPTS solution. Both collections were taken in 1.5 ml microtubes and stored at -18ºC for later analysis.

2.2.4 Quantification of the sucrose concentration of the phloem sap

To quantify the volume of exuded phloem + xylem sap mixture and xylem sap, an aliquot of 20 µl from each sample was diluted in 1 ml tris-HCl (100 mM, pH 7.2) and absorbance at 402 nm was measured with a spectrophotometer (Shimadzu, Japan). These absorbance values were compared with the absorbance value of standard EDTA + HPTS solution to determine the volume of the exudate. Net phloem sap volume was determined by subtracting xylem exudation from the mixture of phloem+xylem exudates. The loss of EDTA + HPTS solution via xylem recirculation was regarded as negligible, as was

confirmed through UV lamp observation of HPTS fluorescence in pedicel cut sections.

Sucrose of the collected samples was hydrolysed through invertase (EC 3.2.1.26, 300 U mg^{-1}, Roche, Germany) in trisodium citrate buffer (320 mM, pH 4.3). The amount of glucose was determined enzymatically using an assay kit, Glucose CII (Wako, Japan). Phloem sap sucrose was calculated by dividing sucrose content of the sap by the estimated phloem sap volume.

2.2.5 Analysis of carbohydrates and organic acids

Fruit samples were freeze dried and ground in a mortar with a pestle. About a 100 mg sample was boiled in 80% ethanol for 2 hours at 85ºC. The extracts were filtered through Whatman GF/F filter paper (25 mm) and the filtrates were dried in a rotary evaporator under vacuum. The extracts were dissolved in 10 ml deionised water.

An aliquot of sample was passed through ion-exchange resin column of Amberlite BM-3 for sugar analysis. Samples 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, 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, USA) 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 along 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) set at 210 nm. Phosphoric acid (0.1 %) was used as the 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, USA) in 1 ml of 0.2M Na-acetate buffer (pH 4.5) at 37ºC overnight. The glucose contents were determined enzymatically using Glucose assay Kit, Glucose CII (Wako) and starch contents were estimated.

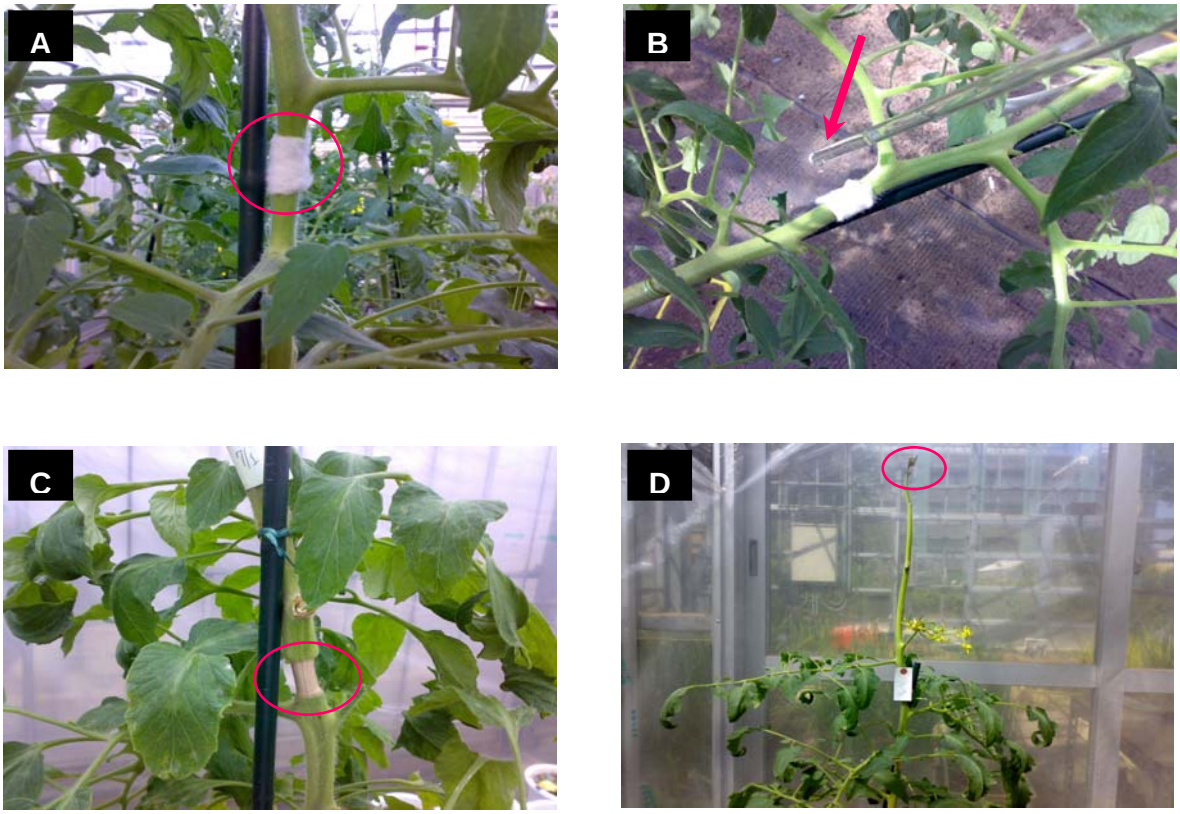


Fig. 1. For heat girdling of plant stem, a piece of cotton was wrapped around the stem (A), and hot water (90ºC) was poured upon with a glass funnel (B). C. Heat-girdled plant stem. Photo (D) shows that only the apical part of the plant was left growing and all the leaves emerging above the second truss were severed off.

Fig. 2. The cut pedicel is being washed with EDTA solution for 2 hours (photo on top). Collection of phloem sap in EDTA + HPTS solution from the cut end of tomato pedicel. The collection vial is sealed and attached to the abscission zone of the pedicel with Parafilm to stop evaporation of the solution. Red arrow indicates heat-girdled area on the pedicel. (photo on bottom).

2.3 Results

2.3.1 Fruit growth and solid content accumulation

When fruit fresh weight, cross-sectional area (fruit size) and fruit dry weight (DW) were plotted against the LFRs, they showed a saturation curve. Fruit fresh weight was low (40 g) at the lowest leaf to fruit ratio (LFR), increased gradually as LFR increased until 1 (80 g), henceforth no obvious change was observed (Fig. 3A). Fruit size also showed similar result to various LFRs (Fig. 3B). The effect of different source-sink ratio was more pronounced in the fruit dry weight (DW). DW increased only under low source-sink ratios from 3.5 to 7 g in the range of 0.2 to 1, and further revealed no apparent change (Fig. 3C). In contrast, percent dry matter increased linearly from 8% to 11% indicating high correlation (r=0.942) with LFR (Fig. 3D).

2.3.2 Soluble solids accumulation

Positive correlations were observed between the LFRs and the total soluble sugars (r=0.890), as well as the organic acids (r=0.943) of fruits. Total soluble sugars (glucose, fructose, and sucrose) increased from 2 to 3% and organic acids (citric, malic, and maleic acids) from 7 to 11 $mg.g^{-1}$ fresh weight, as LFR was enhanced from 0.2 to 3 (Fig. 4A, C). Unlike soluble sugars, starch content saturated at LFR 1, showing no further increase (Fig. 4B). The sum of sugars and starch content exhibited an increasing trend within the whole range of LFR in this study (Fig. 4D).

2.3.3 Phloem sap collection and sucrose concentration

Phloem and xylem exudates were collected in EDTA + HPTS solution from the pedicel (Fig. 2). Pedicel was heat-girdled to prevent phloem sap exudation and to collect the xylem influx. The volume of phloem sap exudate was quantified by the subtraction of xylem volume from the volume of the first exudate containing both phloem and xylem sap.

The dilution rate of HPTS was determined to estimate net exudation of the sap. HPTS is an apoplastic dye which cannot permeate cell plasma membrane, and its absorption via cut end of the pedicel was negligible, provided that evaporation from the pedicel surface was prevented. This modified approach was successful to differentiate and quantify phloem sap volume, which on average ranged from 10-40 $\mu l\ hr^{-1}$ in the collected plants. Sucrose content of the phloem sap increased from 4% to 7% as LFR increased (Fig. 5C). Dry matter percentage of the fruit correlated positively (r=0.930) with phloem sucrose concentration (Fig. 5A). Also significant correlation (r=0.900) between fruit total soluble sugars and phloem sucrose was found (Fig. 5B).

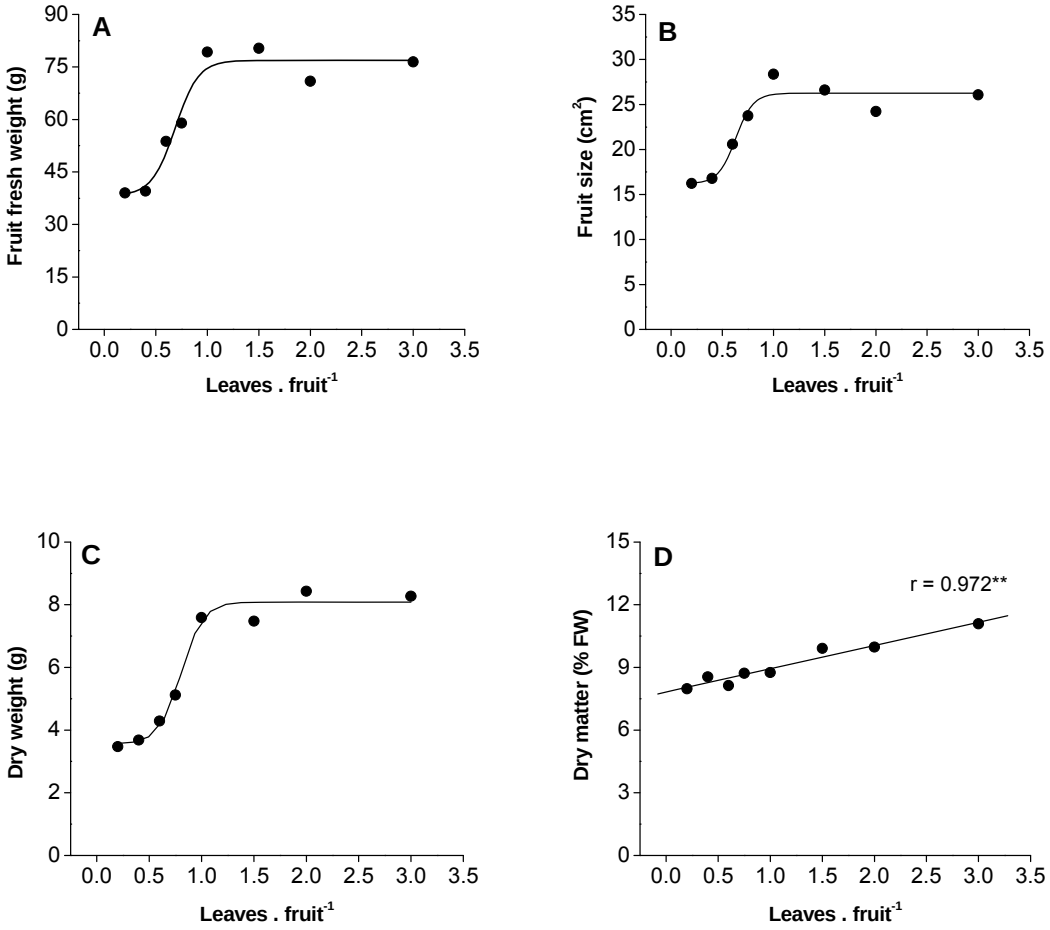


Fig. 3. Relationship of various leaf to fruit ratios with the fruit fresh weight (A), fruit size (B) fruit dry weight (C) and dry matter content (D). Each plot is mean value. The number of replicates are n=4 for LFR of 1/5 and 2/5, n=9 for the other treatments. ** Significant at P<0.01.

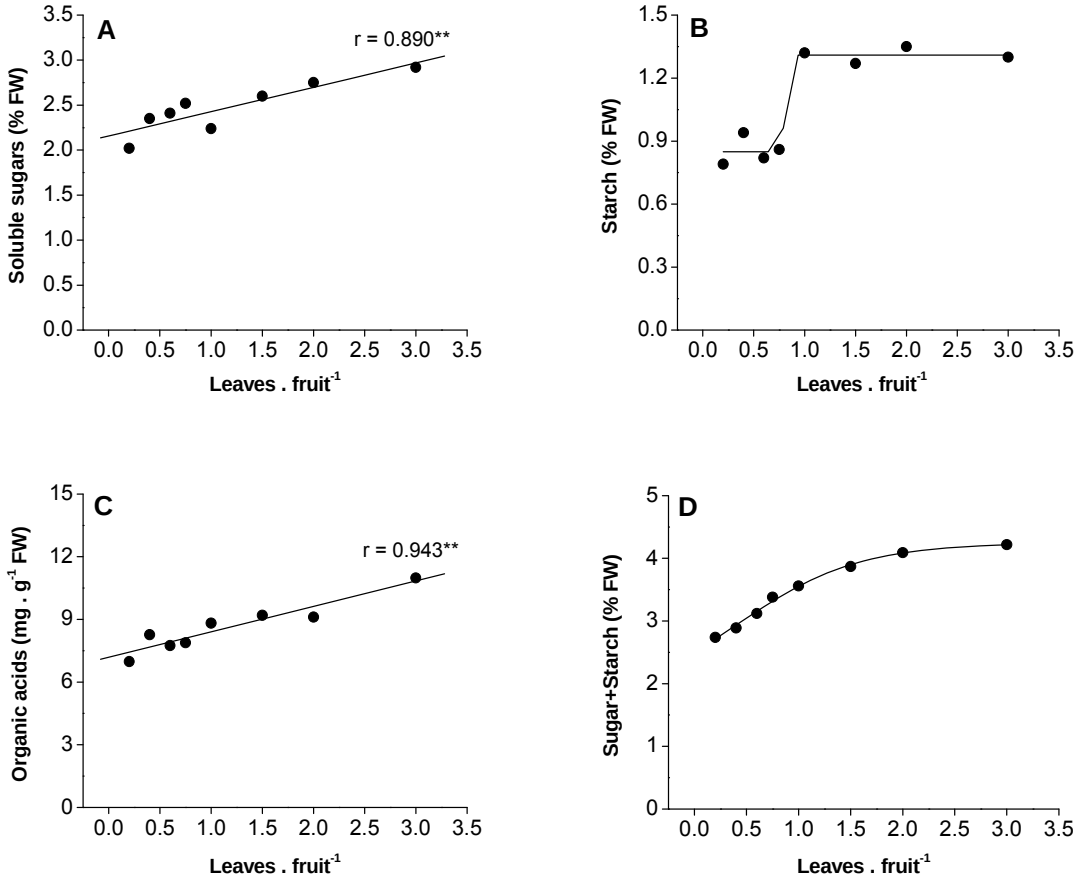


Fig. 4. Effect of various leaf to fruit ratios on the content of soluble sugars (A), starch (B), organic acids (C) and the sum of soluble sugars plus starch (D). Each plot is mean value. The number of replicates are n=4 for LFR of 1/5 and 2/5, n=9 for the other treatments. ** Significant at $P<0.01$.

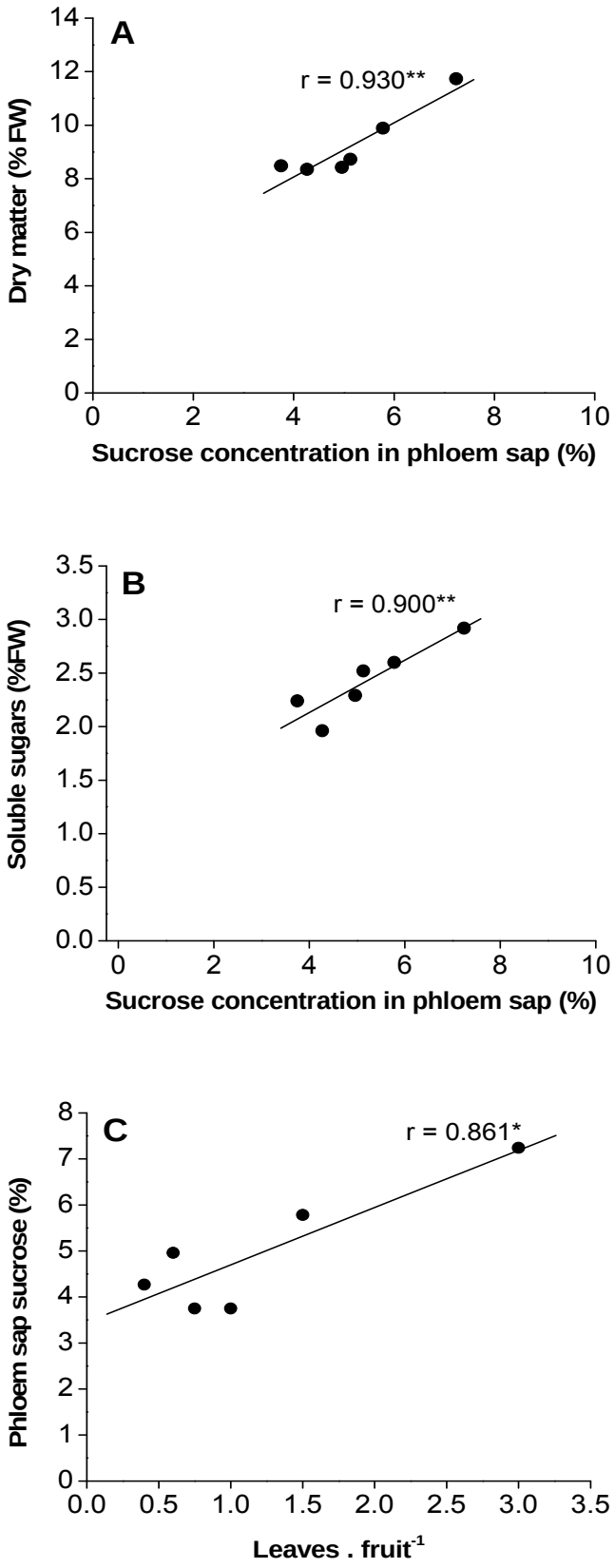


Fig. 5. Relationship of the phloem sap sucrose with fruit dry matter content (A) and soluble sugars content (B). Each plot is mean value. The number of replicates are n=3, 5, 6, 4, 5, and 4 for LFR of 0.4, 0.6, 0.75, 1, 1.5, and 3 respectively.

*‘** Significant at P<0.01 and P<0.05 respectively.

2.4 Discussion

2.4.1 Sink dependent regulation of fruit dry weight

Fruit fresh weight, estimated fruit cross sectional area and fruit dry weight increased steadily as LFR increased up to 1, but further change was very small (Fig. 3A-C). Fruit dry weight increased remarkably by more than 100% from 0.2 to 1 LFRs, but no apparent change was observed from 1 to 3 LFRs (Fig. 3C). Lack of an increase at LFRs higher than 1 indicated that the amount of solids accumulated in a fruit is not dependent on source/sink ratio, but is mostly regulated sink-dependently. The result indicated a saturation type response of fruit dry weight to increasing source-sink ratio. Sink dependent regulation of dry matter accumulation has been observed earlier. Heuvelink and Buiskool (1995) reported dry matter allocation to a truss as a function of the number of fruit per truss in tomato. Marcelis (1993b) observed that high fruit load in cucumber increased cumulative fruit growth at the expense of vegetative growth. Also, the daily dry matter allocation to fruit revealed a saturation response to the total fruit weight on a plant. In cantaloupe, Valantin et al. (1999) observed saturation of the increase in fruit dry weight at the source/sink ratio of two fruits per plant.

Import and accumulation of carbon in a developing sink may depend on the process of sucrose unloading as well as sucrose concentration of the phloem sap. Dorais et al. (1999) hypothesized that the import rate of sucrose in fruit is determined by the carbon utilization of the sink. The rate of phloem unloading is supposed to be regulated by the activity of sucrose metabolizing enzymes (Dorais et al., 1999), sucrose transporters (Kuhn et al., 2003; Hackel et al., 2006), and post-unloading sugar metabolism and compartmentation (Wang et al., 1993; Ho, 1996). Therefore, sucrose uptake from the phloem was presumably limited by the unloading processes governed by the fruit growth. The results also indicated that one leaf per fruit was enough to provide the required carbon

for the maximum fruit growth, and increasing LFR may not improve dry matter accumulation into fruit beyond its threshold level.

2.4.2 Source dependent regulation of solid content of fruits

Unlike fruit dry weight, dry matter content as well as soluble solid content on a fresh weight basis increased linearly by 40 to 50% as increased source-sink ratio investigated in this study (Fig. 3D, 4A, C). This data implies that dry matter content on fresh weight basis is dependent on leaf area per fruit and therefore is source dependent. High leaf area per fruit was observed in tomato genotype having high fruit dry matter content (Hewitt and Stevens, 1981). Similarly, dry matter percentage and sucrose contents of the mango flesh were observed to increase significantly by high LFR (Lechaudel et al., 2005).

While the concentration of sugars and organic acids showed a linear correlation to LFRs, starch content showed saturation above LFR of 1 (Fig. 4B). Starch accumulation may be determined by the balance between its synthesis and degradation, which may be regulated enzymatically. The accumulation of starch was presumably saturated due to the high concentration of sugars in the fruits, but not due to the high LFR itself. Luengwilai et al. (2010) pruned tomato plant to two fruits per truss and observed no significant change in fruit starch content and its synthesis because of changing LFR.

All of these results are in agreement with this hypothesis that, while total fruit dry weight, which may be regulated by the phloem unloading process, is determined sink-dependently, solid contents on a fresh weight basis is determined source-dependently by the sucrose concentration of the phloem sap loaded in the photosynthesizing leaves. Sucrose synthesized in leaves is translocated to fruit via the phloem along with water. Therefore, increase in sugar content of the sap at high LFR would directly influence the proportion of sucrose transport to water transport into fruits, and thereby influence the final

sugar concentration of fruit. To confirm such relationship between fruit solid content and phloem sucrose, we attempted to measure the phloem sucrose.

2.4.3 Estimation of phloem sap sucrose by modified EDTA-method

To confirm the relationship between the solid content of fruits and phloem sucrose, we attempted to collect the phloem sap from the pedicel of tomato fruits and to quantify its sucrose concentration. The method was based on the EDTA method (King and Zeevaart, 1974) modified by Araki et al. (1997) for tomato. Araki et al. (1997) successfully collected the exudate from the phloem of intact pedicel, but they did not quantify the sucrose concentration of the exudate, since the volumes of phloem exudates were unknown. In grape (Lang and Thorpe, 1989) and tomato (Guichard et al., 2005), the subtractive method employing heat-girdling was used to separately quantify the phloem flow and the xylem flow, estimated by the direct measurement of the volume growth of fruits as attached to the plant. In this work, we combined the subtractive method along with the EDTA method to collect and estimate the volume of the phloem sap. We supposed that estimated volume of the exudate, calculated from the dilution rate of HPTS added to the sampling solution, before and after the heat-girdling give us an estimate of net phloem sap collection.

Sucrose concentration of the phloem sap was calculated as sucrose exudation in the sampling solution divided by the estimated phloem sap volume. To avoid the possibility of the contamination of sucrose leakage from apoplasts and sucrose hydrolysis by acid invertase released from the wounded cells (Amiard et al., 2004), we discarded the initial two-hour exudate, as van Bel and Hess (2008) reported that the collection after one-hour incubation in EDTA solution was practically free from sugar leakage. In addition, sucrose was collected in neutral buffer to inhibit sucrose hydrolysis by acid invertase. On average, a sample of 10-40 µl phloem sap was collected in various treatments. Phloem sucrose ranged from 3 to 7% depending on the treatments. These values are in agreement with the

estimation for phloem sap concentration in tomato by Ho et al. (1987) and Plaut et al. (2004) in their control plants, which was assumed similar to the concentration of sucrose (Ho et al., 1987).

From the cut pedicel, the observed ratio of exudation for phloem to xylem was lower than the estimated ratio of transport for phloem to xylem in intact plants reported by Ho et al. (1987) and Plaut et al. (2004). The hydraulic resistance to xylem flow is reported to occur within the fruit pericarp (Malone and Andrews, 2001); therefore, the ratio of the exudation of phloem to xylem can vary once fruit is severed from the pedicel.

Positive correlations were observed between phloem sucrose concentration and percentage of fruit dry matter per fresh weight, as well as the concentrations of total sugars (Fig. 5A, B). In studies of tomato grown under saline-stress or high nutrient EC, higher concentrations of dry matter was attributed to the higher concentration of phloem sap sugars rather than higher relative phloem water flow (Ho et al., 1987; Plaut et al., 2004). These data suggest that dry matter and soluble sugar concentrations are mainly determined by the phloem sap sucrose concentration, not only under stressed conditions but also under non-stressed conditions. The altered sucrose concentration of the phloem sap may be due to the shift of balance between the water demands by the fruits and the capacity of source leaves to load sucrose into the phloem. Assuming that the phloem is the main rout of the water transport into fruits (Ho et al., 1987; Araki et al., 2004; Plaut et al., 2004; Guichard et al., 2005), high fruit load would accelerate water export from the source leaves through the phloem. At a higher rate of water export, the rate of sucrose loading at the source leaves may delay as compared with the faster water export, thereby decreasing the sucrose concentration of the phloem sap. Also, under low fruit load, the competition for assimilates between source leaves will presumably be lowered, and this may lead excessive accumulation of carbohydrates in source leaves. High source / sink ratio was reported to

increase leaf area (Heuvelink and Buiskool, 1995) and leaf mass per unit leaf area (Bertin and Gary, 1998; Heuvelink and Buiskool, 1995). Such potential alteration may also affect sucrose concentration of the phloem sap. The evaluation of leaf area, carbohydrate content, or photosynthetic activity of source leaves in response to increased LFR would be necessary to reveal the underlying processes which lead to increase the sucrose concentration of the phloem sap. Since excessive increase in LFR conversely affects fruit yield, understanding of the mechanism would be beneficial for the improvement of fruit quality without decreasing fruit yield.

Our prediction is based on the previous observations that most of water transport into fruit is via the phloem, and under the conditions that condensation effects of water loss as transpiration flow and xylem backflow are minor. However, recent papers raised a question whether the phloem is the main route of the water transport into fruits and the xylem water flow is minor. Studies in tomato (Malone and Andrew, 2001) and grape berries suggested the functional xylem connection between fruits and plants throughout the development of the fruit (Bondada et al., 2005; Keller et al., 2006; Chatelet et al., 2008; Tilbrook and Tyerman, 2009). Moreover, Windt et al. (2009) measured intact water flow in the truss stalk by nuclear magnetic resonance flow imaging and claimed that most of water in the tomato truss is transported through the xylem, but not phloem. However, the data also suggested that most of xylem water transported in the truss did not enter the fruits, but circulated within the truss returning back to the plant. Although the water relations of phloem and xylem flow are not fully understood, the present data validated the importance of the concentration of phloem sap sucrose and also supported the current hypothesis that water is mainly transported via the phloem into tomato fruits.

It should be noted that the correlation between phloem sap sucrose and fruit dry matter concentration was still found at LFR higher than 1, at which dry matter

accumulation per fruit saturated. The optimum source-sink ratio is usually set as three to five fruits per truss in crop production considering the saturation of the increase in fruit dry weight at the higher LFR. However, the present data implies that source-sink ratio higher than LFR of 1 has a positive effect on fruit quality. Therefore, it would be still possible to improve fruit quality by increasing the concentration of the phloem sap by manipulating source-sink balance. Although, we studied 3 weeks old green fruit, the stage when fruit develop rapidly, for organic contents and phloem sap analysis. As tomato fruits accumulate most of its dry matter at rapid stage of development (Ho et al., 1983), therefore, at this stage, the relationship between dry matter and phloem sucrose concentration can better explain the final status of solid in fruit.

In summary, the present work showed that, while elevating the source-sink ratio, LFRs higher than of 1 have a negligible effect on fruit growth, but can increase fruit solid content as well as the sucrose concentration of the phloem sap. The optimum source-sink ratio is usually set as three to five fruits per truss in crop production (Kang et al., 2009), and decreasing the number of fruits per truss is regarded as having no advantage, considering the saturation of fruit growth at higher LFR. However, it could be possible to improve fruit quality by manipulating source strength, such as by increasing the source leaf area per fruit, or elevating photosynthetic activity, light environment, and ambient carbon dioxide levels.

2.5 Summary

Regarding the previous observations that more than 90% of the water is translocated via the phloem into a fruit in tomato, soluble solid content of fruits may be strongly influenced by the sucrose concentration of the phloem. In this study, we evaluated the relationship of fruit soluble solid contents and phloem sap sucrose concentration in tomato at various source-sink ratios. Fruit dry weight increased linearly (r=0.930) from 3.5 to 7 g when the leaf / fruit ratio (LFR) was increased from 0.2 to 1, indicating source dependent regulation of dry matter accumulation. However, it did not change beyond the LFR of 1, indicating that dry matter accumulation was not affected by the source. In contrast, fruit percent dry matter, total soluble sugar and organic acid content increased linearly within the whole range of LFR from 0.2 to 3 (r=0.972, r=0.890, r=0.943, respectively). Using these plants, phloem sap was collected from the cut end of the pedicel in EDTA solution. The sucrose concentration of the collected phloem sap showed a positive correlation to the percentage of fruit dry matter (r=0.930). Fruit total sugars also correlated positively with phloem sucrose concentration (r=0.900). These data suggested that, while dry matter accumulation per fruit is not affected by the source above LFR of 1, the content of soluble sugars and dry matter on a fresh weight basis is determined source dependently via the sucrose concentration of the phloem sap.

Chapter-3

3. Impact of saline-alkali stress on the accumulation of solids in tomato fruit

3.1 Introduction

In fleshy fruit like tomato, where dry matter accounts for less than 10% of the fresh weight (Davies and Hobson, 1981), the improvement of solid content is of prime importance for quality improvement. High solid content (SC) of fruit may enhance the consumer demand and market value of the fresh produce, besides increasing its processing efficiency.

Solid accumulation in 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 SC (Mitchell et al., 1991a, b; Mizrahi et al., 1988; Renquist and Reid, 2001; Patane and Cosentino, 2010), with high sugar and organic acid contents (Ho et al., 1987; Gao et al., 1998; Sakamoto et al., 1999; Plaut et al., 2005; Sato et al., 2006; Saito et al., 2008).

Soil salinity and low availability of fresh water for irrigation are serious problems 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 yield may decrease but fruit SC is reported to increase which may compensate for low yield. Literature regarding SC increase in tomato fruit 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 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. Such soils are termed as sodic soil, saline-alkali, or alkali soils and are even larger in area than saline soils in the world. The

world has an estimated land surface of about 13.2 x 10^9 ha, out of which only 7 x 10^9 (53%) are arable, but only about 22% (1.5 x 10^9 ha) of the arable land is under cultivation. Saline-alkalinity affects 37% of the cultivated land, whereas 22.5% is affected by salinity. Both saline and sodic soils are spread in more than 100 countries of the world (Tanji, 1996), whereas, saline-alkali soil is particularly prevalent in Asia, Australia and the Pacific region (FAO). Since saline stress (high EC) is known to increase the accumulation of solid, especially in tomato (Ho et al., 1987; Mitchells et al., 1991a; Yin et al., 2010), but it is not known whether saline-alkali stress (high EC and pH) can also influence fruit solid accumulation. However, this problem has not been studied properly and no data is available for tomato. Therefore, it is necessary to understand the influence of saline-alkali stress on the solid content of fruit, especially in tomato. Such understanding would help us to improve fruit quality as well as broaden the existing knowledge for the genetic improvement of new varieties to exploit such stress conditions.

The scanty available literature showed that when wheat plants were irrigated with bicarbonate rich water, soluble carbohydrate contents of grain increased and protein contents decreased in comparison to control plants (Paliwal et al., 1975). Addition of $NaHCO_3$ (0-20 mM) to nutrient solution increased organic acid content in the roots of cereal crops, but decreased dry weight of their roots and shoots (Alhendawi et al., 1997). Bialczyk et al. (1996) reported that incorporation of $KHCO_3$ at very low level (6 mM) in the rooting medium of tomato plants increased the fruit yield and contents of hexose and organic acids much higher than control.

Saline-alkali and saline stresses are regarded two different stress conditions (Yang et al., 2007, 2008a, b), but the influence of saline-alkali stress on fruit SC has never been studied either in hydroponic culture or soil medium for tomato crop. In this experiment, the impact of saline-alkali stress on the accumulation of solids in fruits was assessed in tomato.

3.2 Materials and Methods

3.2.1 Plant materials

Tomato seed of 'House Momotaro' were obtained from Takii (Kyoto, Japan). During summer 2009, seed 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, Japan) and granulated soil (Engei Baido, Kureha, Tokyo) in equal proportion.

All the plants were grown under sunlight in a glasshouse with proper ventilation to avoid high temperatures. 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 plants was severed at fruit set to maintain a proper source-sink balance. Upon anthesis, the first flower was pinched off and the subsequent two flowers were sprayed for one time with synthetic plant growth regulator 'tomato tone' (Ishihara, Japan) for uniform setting.

3.2.2 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) without any salt. After one week, plants were subjected to four saline-alkali treatments (30, 60, 90 and 120 mM) or 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 half-strength Otsuka nutrient solution twice a week, usually one day prior to the salt treatments.

Control plants were fertilised twice a week with half-strength Otsuka nutrient solution. These plants were irrigated with 1L tap water when required.

3.2.3 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. Every week, one time each after salt and nutrient solution application, i.e. twice a week, pH and EC of the leachate was 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, the 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, 5-6 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 -18 ºC.

3.2.4 Analysis of carbohydrates and organic acids

Fruit samples were freeze-dried and ground in a mortar with pestle. About 100 mg samples were boiled in 80% ethanol, for 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) solution 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) 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 0.2M 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 in the glasshouse, each treatment having 10 replications. The data was analysed according to one-way analysis of variance (ANOVA). The means were compared by using Student-Newman-Keuls test in case of significant result.

3.3 Results

3.3.1 Soil leachate pH and EC

pH of the soil leachate indicates that pH increased to 8 in 90 and 120 mM treatments after two weeks of $NaHCO_3$ application (Fig. 6A). Later on, pH remained higher than 8 until the end of the experiment. In 60 and 30 mM treatments, pH reached to 8 only at the later half of plant growing period. In contrast to $NaHCO_3$ treated plants, pH of the control plants (0 mM) remained relatively unchanged between pH 6 and 7, throughout the plant growth. The mean pH was 6.6 in control treatment, while pH was 7.5, 7.9, 8.2, and 8.3 in 30, 60, 90, and 120 mM treatments respectively (Fig. 6 C).

Soil leachate EC for 90 and 120 mM treatments were much higher than control treatment (Fig. 6B). In control treatment, EC of the soil leachate decreased gradually from 4 to 1.5 dS m^{-1} at the end of growing period, while EC increased to 5 and 6 dS m^{-1} at 90 and 120 mM treatments respectively, at the end of growing period. Mean values show EC of the leachate was below 4 dS m^{-1} at 30 and 60 mM treatments but higher than 4 dS m^{-1} at 90 and 120 mM treatments (Fig. 6 D).

3.3.2 Fruit growth and its total solids

Tomato fruit size (FS), estimated from cross sectional area, and fresh weight (FW) was not significantly decreased by saline-alkali treatment between 0 to 90 mM stress treatments. Fruit size and FW was decreased only at 120 mM treatment (Fig. 7A, B). FS in control plants was 42 cm^2, it remained 40 and 36 cm^2 in 90 and 120 mM stress treatments respectively (Fig. 7A). Likewise, fruit FW within the range of 0-90 mM saline-alkali stress was 185 g - 170 g, but it decreased to 150 g at 120 mM (Fig. 7B).

Saline-alkali treatments up to 120 mM had no significant influence on fruit dry weight (DW) accumulation in comparison to the control treatment. In control, DW was

12.5 g, which increased to 14 g in 60 and 90 mM treatments (Fig. 8A). Unlike DW, fruit dry matter content on a fresh weight basis was significantly increased at above 30 mM treatment. Minimum dry matter content was observed in control plants (6.8%), while the maximum of 8.5% was recorded in 90 and 120 mM treatments (Fig. 8B).

3.3.3 Soluble sugar and organic acid accumulation

Glucose and fructose were the major accumulated sugar in ripe fruits. Both sugars were accumulated in almost equal proportion by all treatments. Glucose concentration was 0.9 % in the control, which increased gradually as salt concentration increased reaching to 1.4 % at 90 mM treatment. The concentration of glucose decreased at 120 mM salt level (Fig. 9A). The content of fructose was the lowest in control (1 %) and the highest in 90 mM treatment (1.4 %) (Fig. 9B).

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. 10A). Starch content of the fruit was very low except at 120 mM where its content was significantly higher than control fruit (Fig. 10B).

TSS on FW basis (Fig. 11A) increased significantly from 2 to 3% in 90 mM treatment as compared with control, but declined at 120 mM treatment. At the other stress treatment, there were no significant differences in the content of total sugar as compared to control. The maximum sugar level at 90 mM was higher than control by 55%.

Citric acid was the major accumulated organic acid (OA) in the ripe fruit, followed by malic and maleic acids. Citric acid content was significantly higher in 90 and 120 mM treatments (25 and 32 $mg \cdot g^{-1}$ FW respectively) than control and 30 mM treatments (19 $mg \cdot g^{-1}$ FW) (Fig. 11B). Malic acid content was not significantly affected in all treatments (2.85 $mg \cdot g^{-1}$ - 3.6 $mg \cdot g^{-1}$ FW) (Fig. 12A). Maleic acid content was very low in fruit and

saline-alkali treatments did not affect it significantly (Fig. 12B).

3.3.4 Correlation between fruit TSS and pH/EC

Fig. 13 (A, B) show the correlation between fruit TSS and soil leachate pH and EC. When TSS was plotted against soil leachate pH, it shows positive correlation ($r = 0.871$). Similar positive relation was revealed with soil leachate EC ($r = 0.795$).

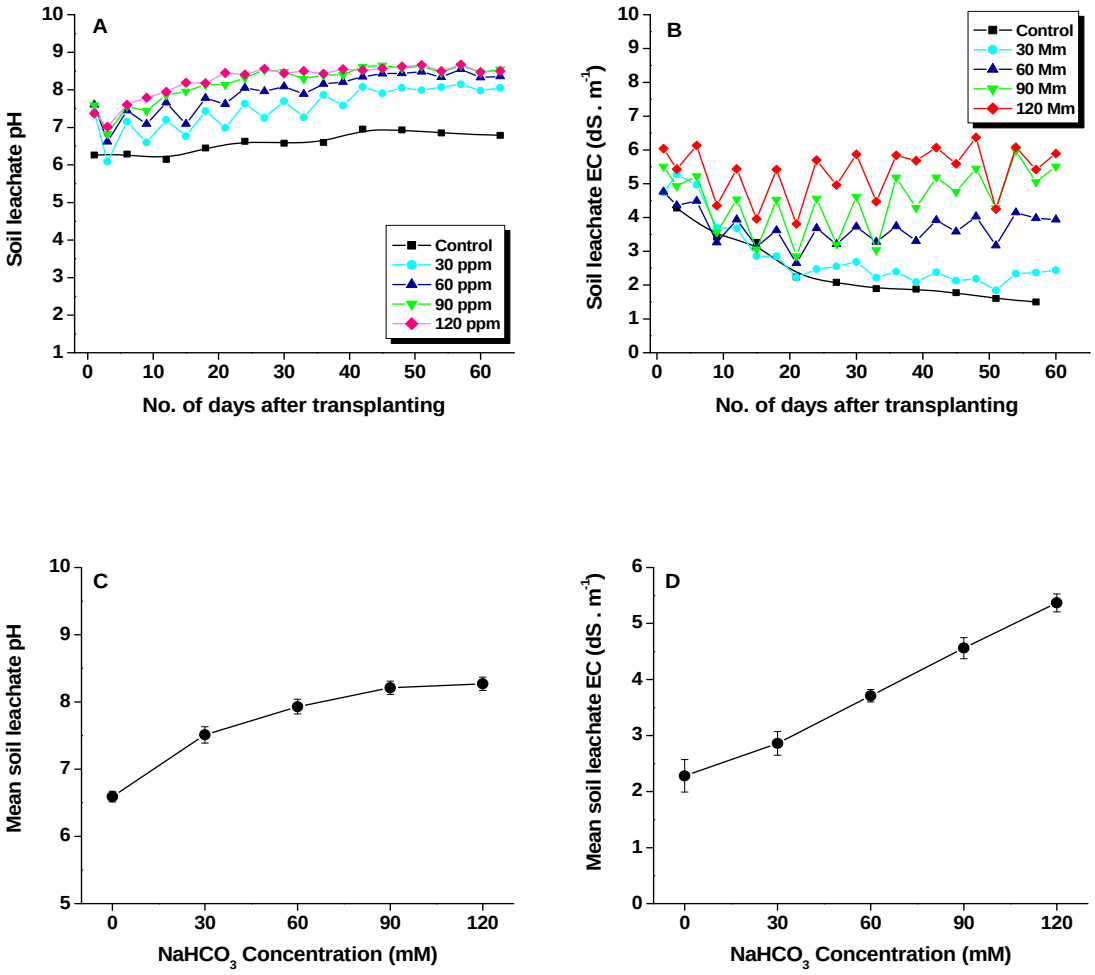


Fig. 6. Effect of saline-alkaline stress conditions (0-120 mM) on soil leachate pH (A) and EC (B) in tomato plants grown in soil compost for the whole growing period. Value of pH and EC were recorded twice a week in leachate collected after salt and nutrient solution application. C and D show mean data for 0-120 mM treatments. Each plot is the mean value of n=10.

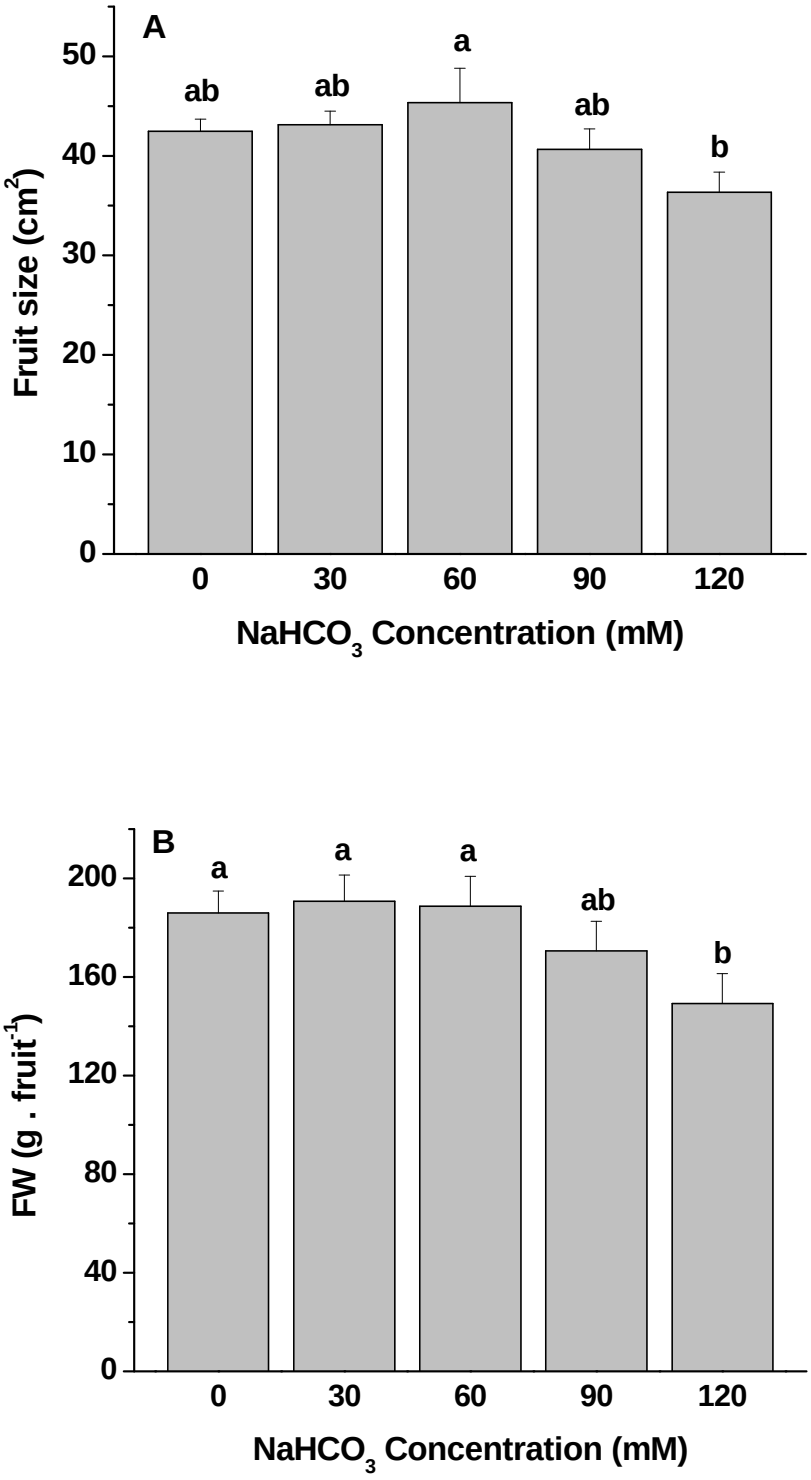
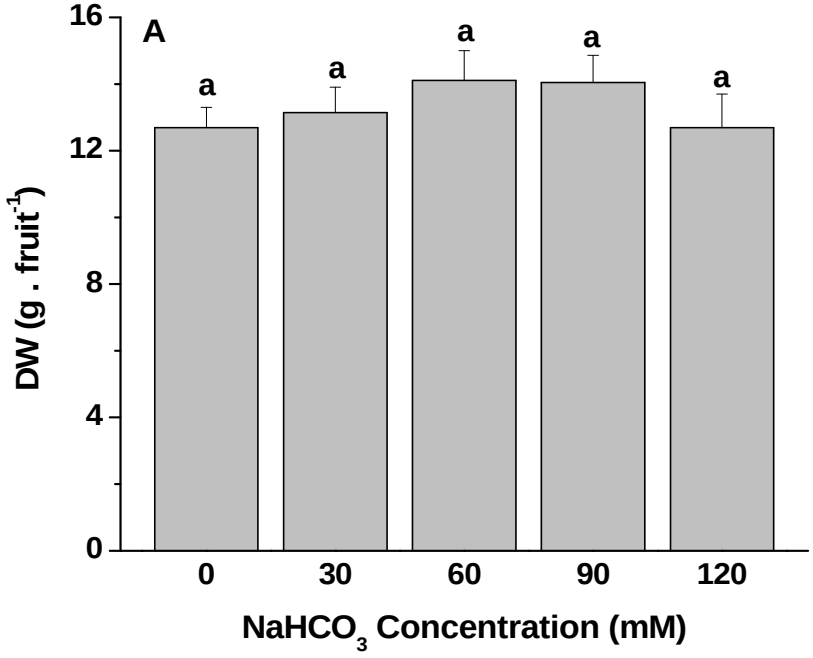


Fig. 7. Effect of saline-alkaline stress conditions (0-120 mM) on the fruit size (A) and fresh weight (B) of tomato grown under glasshouse in soil compost. The bars indicate means ± SE. The number of replicates are n = 7-10. Different letters over the bars show significant difference among treatments ($p < 0.05$) as determined by ANOVA and separated by Student-Newman-Keul's multiple range test.



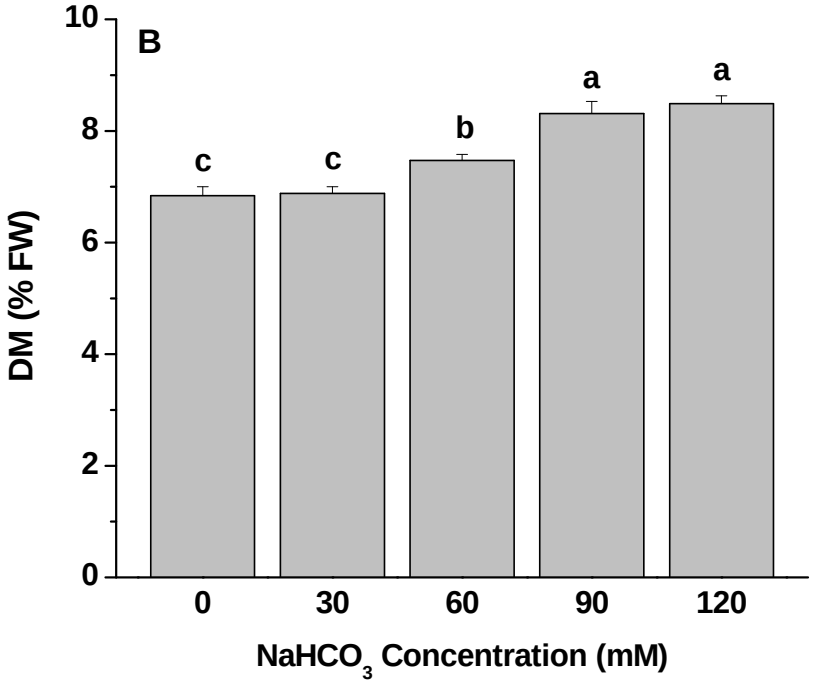


Fig. 8. Effect of saline-alkaline stress conditions (0-120 mM) on the dry weight (A) and dry matter % (B) of tomato fruit grown under glasshouse in soil compost. The bars indicate means ± SE. The number of replicates are n = 7-10. Different letters over the bars show significant difference among treatments ($p < 0.05$) as determined by ANOVA and separated by Student-Newman-Keul's multiple range test.

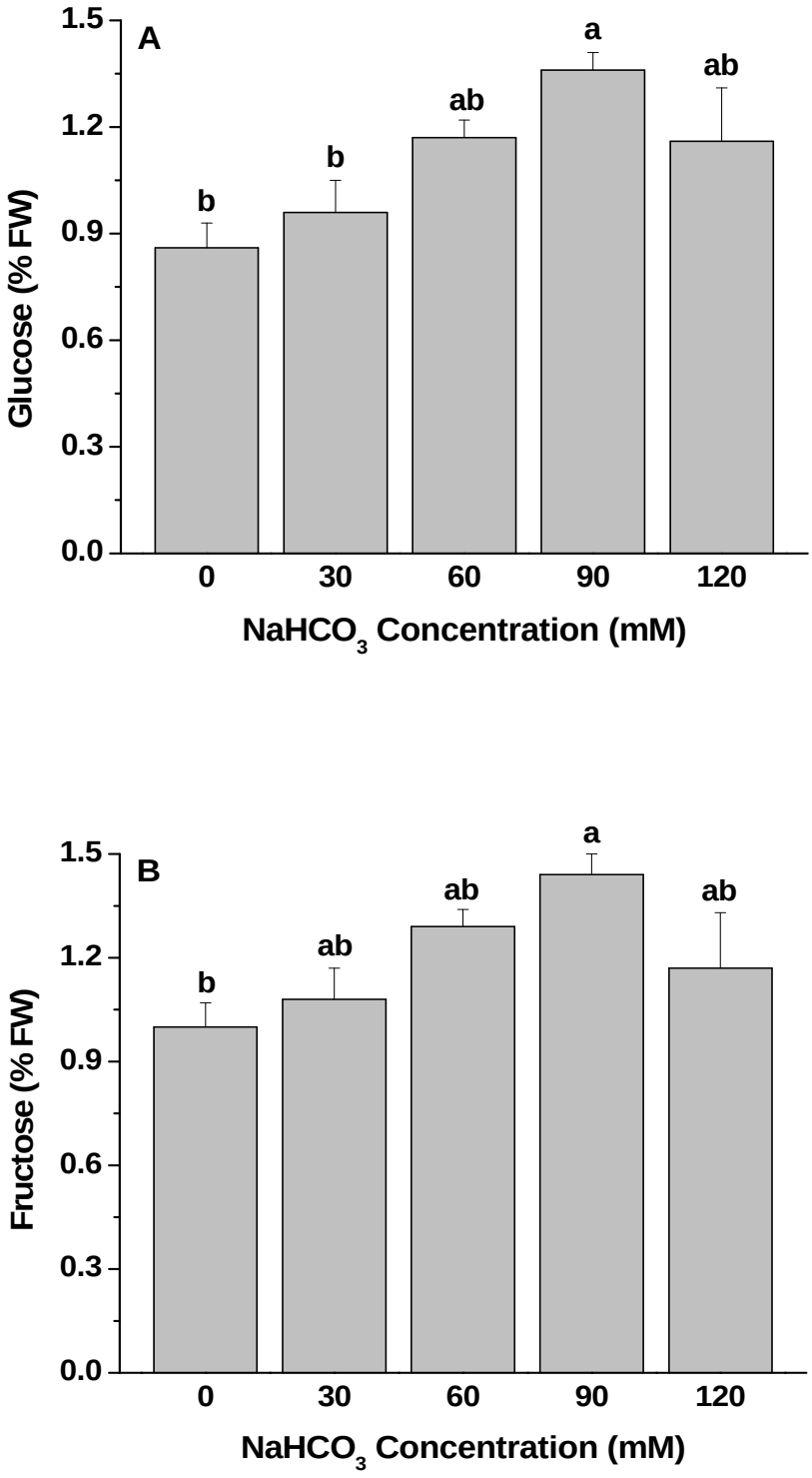


Fig. 9. Effect of saline-alkaline stress conditions (0-120 mM) on the glucose (A) and fructose (B) % of tomato fruit grown under glasshouse in soil compost. The bars indicate means ± SE. The number of replicates are n = 7-10. Different letters over the bars show significant difference among treatments ($p < 0.01$) as determined by ANOVA and separated by Student-Newman-Keul's multiple range test.

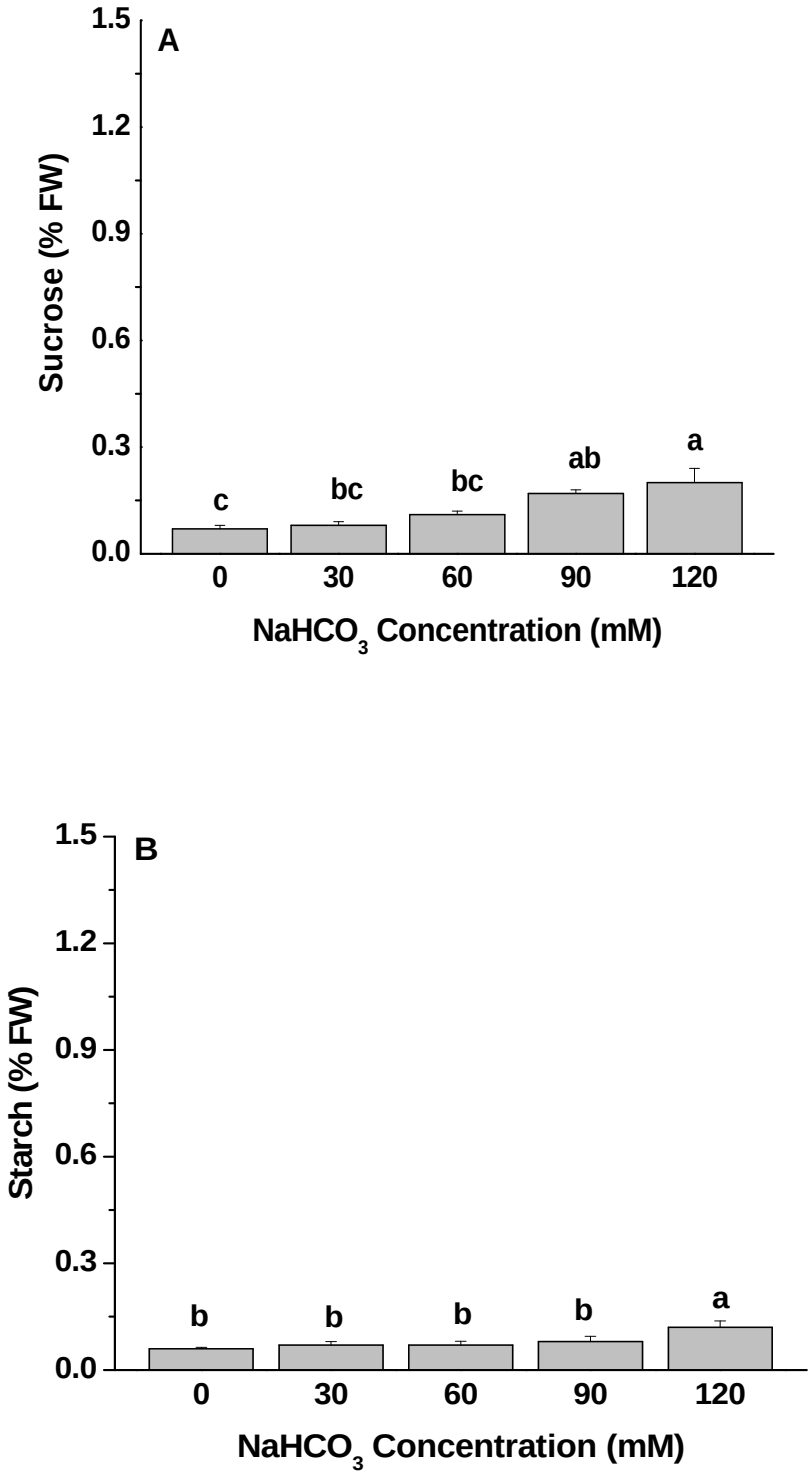


Fig. 10. Effect of saline-alkaline stress conditions (0-120 mM) on the sucrose (A*) and starch (B) % of tomato fruit grown under glasshouse in soil compost. The bars indicate means ± SE. The number of replicates are n = 7-10. Different letters over the bars show significant difference among treatments ($p < 0.01$*, $p < 0.05$) as determined by ANOVA and separated by Student-Newman-Keul's multiple range test.

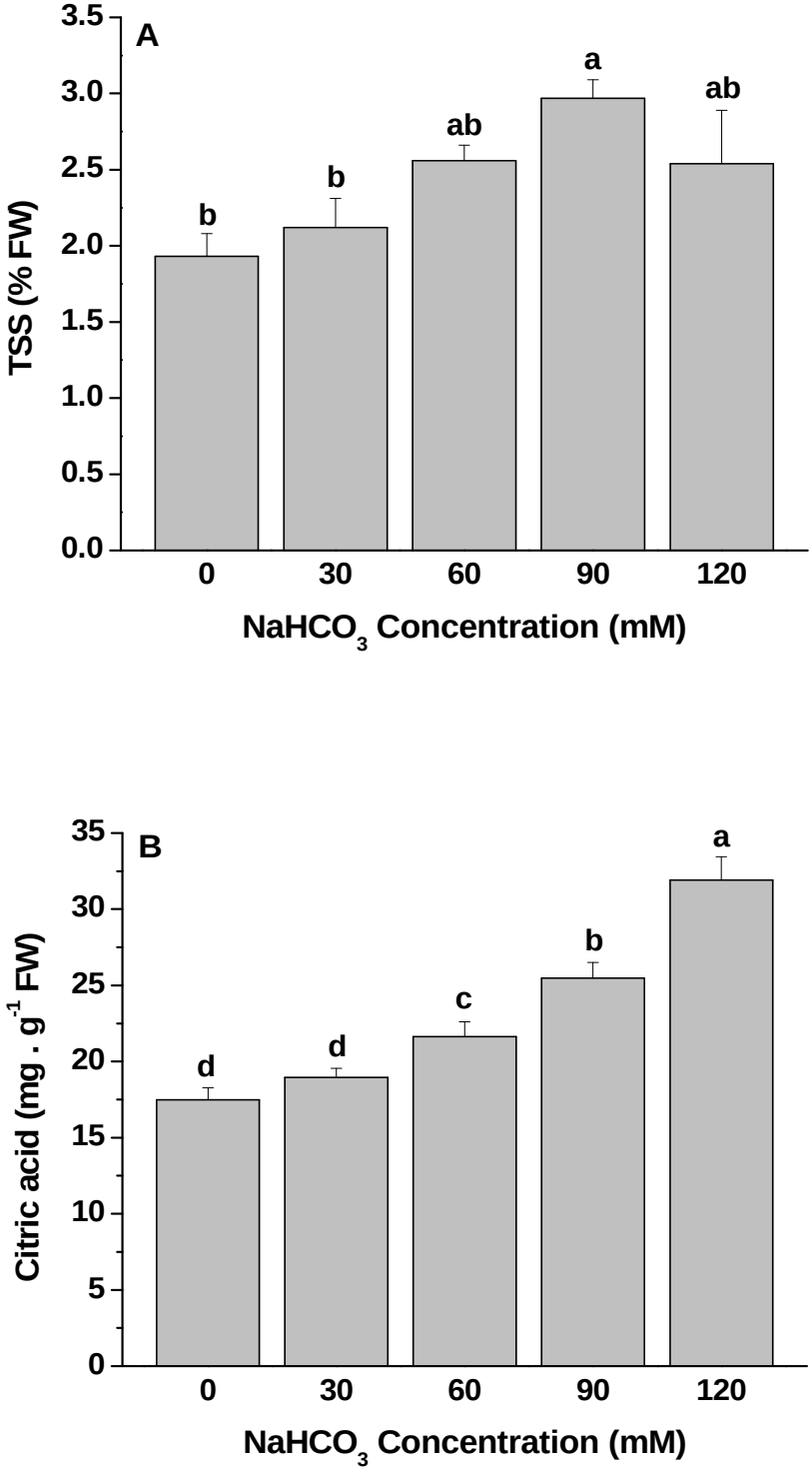


Fig. 11. Effect of saline-alkaline stress conditions (0-120 mM) on the TSS (A) and Citric acid (B) % of tomato fruit grown under glasshouse in soil compost. The bars indicate means ± SE. The number of replicates are n = 7-10. Different letters over the bars show significant difference among treatments ($p < 0.01$) as determined by ANOVA and separated by Student-Newman-Keul's multiple range test.

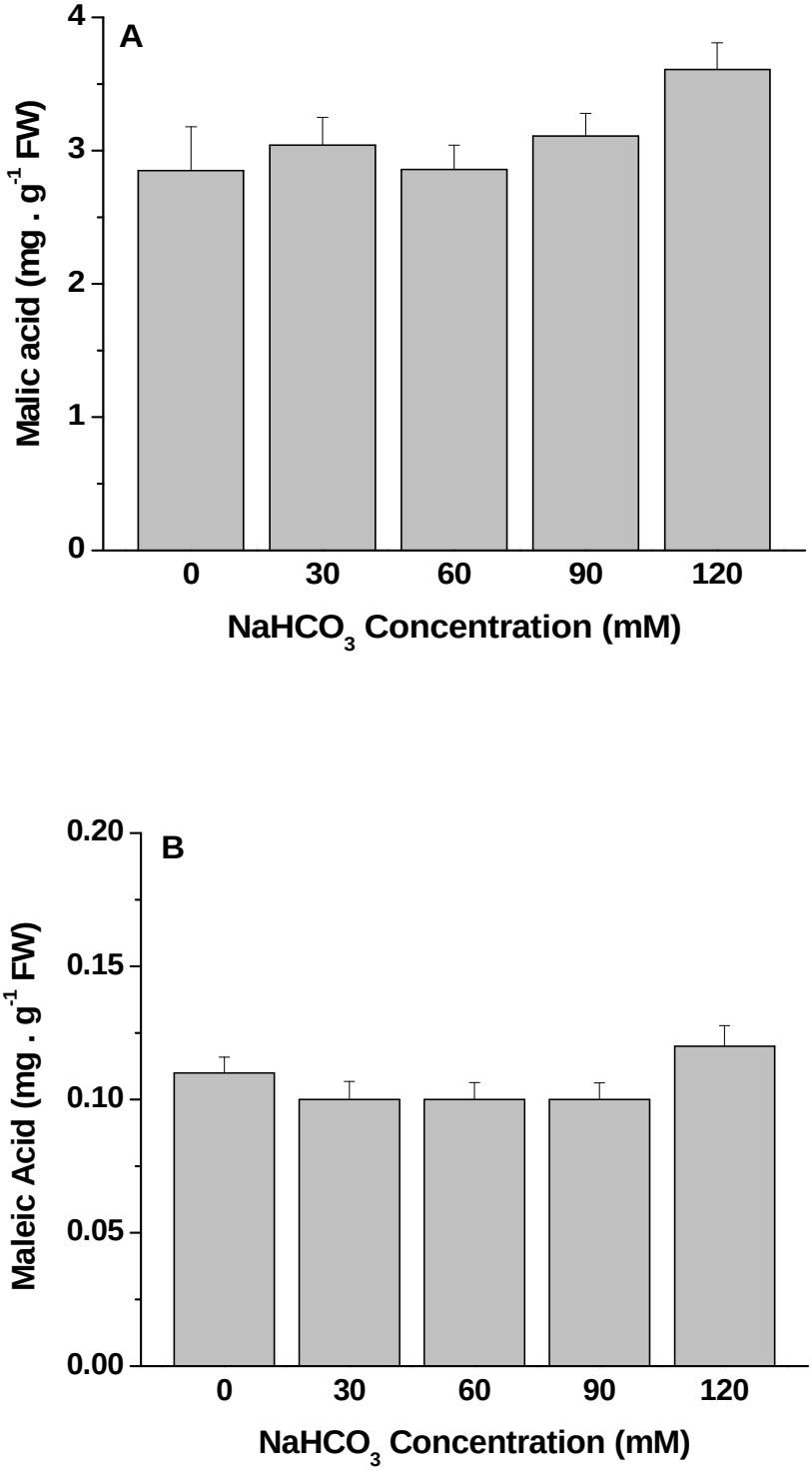


Fig. 12. Effect of saline-alkaline stress conditions (0-120 mM) on the Malic acid (A) and maleic acid content (B) of tomato fruit grown under glasshouse in soil compost. The bars indicate means ± SE. The number of replicates are n = 7-10. The absence of letter above the bars shows the treatments had a non-significant influence as determined by ANOVA.

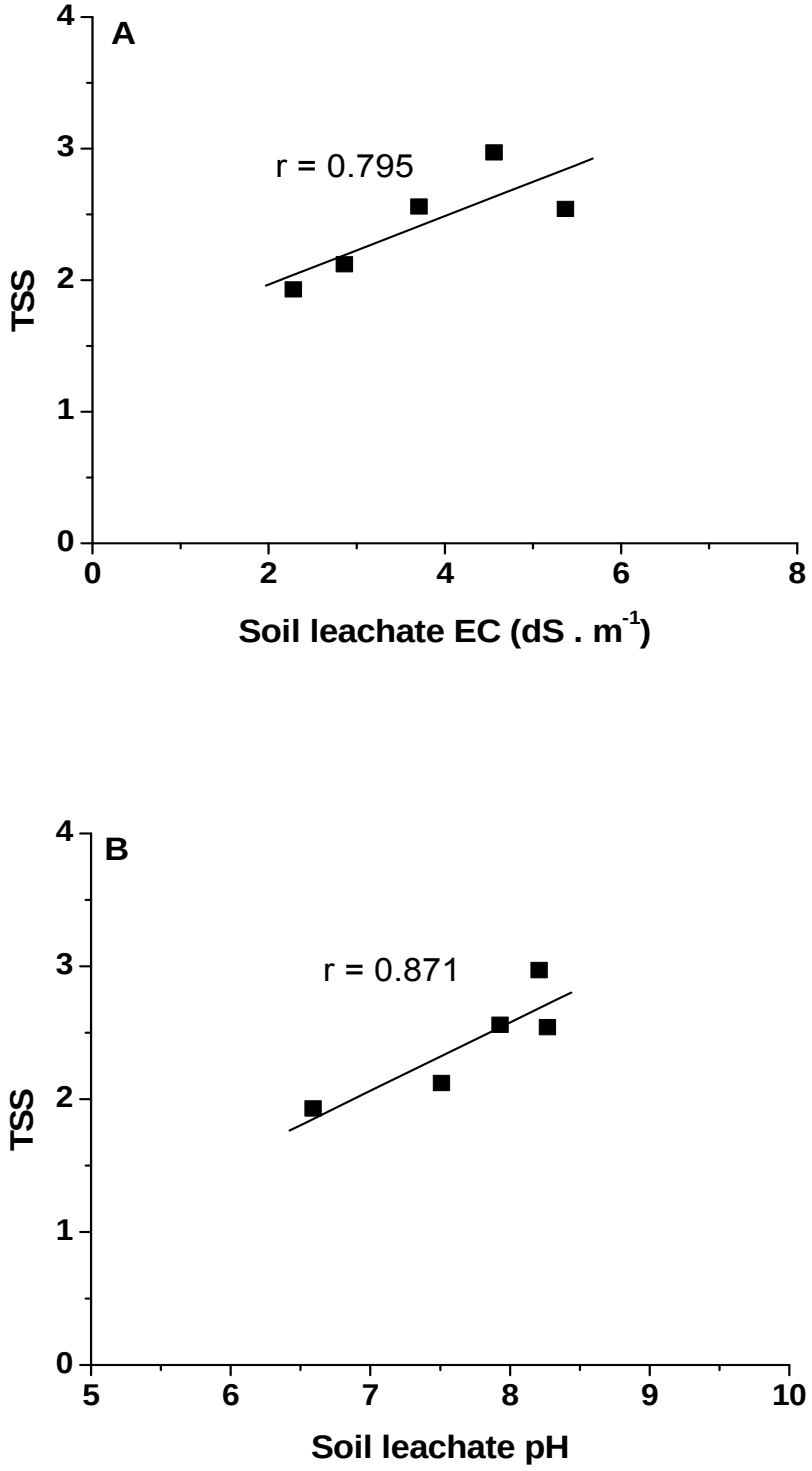


Fig. 13. Correlation curve indicating the relationship between TSS of fruit plotted against EC or pH of soil leachate. Each plot shows mean value (n = 7-10).

3.4 Discussion

3.4.1 Regulation of soil leachate pH and EC

In this study the influence of saline-alkali stress on solid accumulation in tomato fruit was evaluated at the ripe red stage. Sodium bicarbonate salt, when added to the soil, increased the soil pH as well as EC as is indicated by the soil leachate data (Fig. 6A-D). After two weeks of the salt treatments, soil leachate pH reached to 8, especially in 90 and 120 mM salt treatments, while the pH of the soil leachate was within the range of 6-7 in control treatment, with no apparent change. Such high pH, observed in the treated soil leachate, may be due to the presence of sodium bicarbonate salt and especially the bicarbonate ions in the pot soil. $NaHCO_3$ salt when added to the soil may ionise into sodium and bicarbonate ions and thereby influence soil pH. The given equation shows the dissociation of bicarbonate salt into its ions in soil.

$$NaHCO_3 + H_2O \rightarrow Na^+ + *HCO_3^- \rightarrow HCO_3^- + H_2O \rightarrow H_2CO_3 + **OH^-$$

Apart from soil pH, soil leachate EC was also increased by saline-alkali treatments (Fig. 6B, D). In control treatment, soil leachate EC decreased gradually from 4 dS m^{-1} to 1.5 dS m^{-1} until the end of the growth period, while in 90 and 120 mM treatments soil leachate EC increased gradually and remained higher than 4 dS m^{-1}. It may be because of the ionization of $NaHCO_3$ in soil (as shown in the above equation) which may increase the concentration of $*HCO_3^-$/$**OH^-$ and Na^+ ions in the rooting medium, thus culminating in increased EC as well as pH of the soil leachate. This data show that addition of up to 60 mM $NaHCO_3$ to pot soil may strongly increase soil pH but soil EC may remain below the defined threshold level of saline soil (4 dS m^{-1}). Likewise, it appears that addition of bicarbonate salt may increase soil pH to a certain level only, as after 4-5 weeks of salt application there seems no further increase in the pH of the soil leachate in the salt treatments (Fig. 6A).

3.4.2 Fruit fresh and dry weight accumulation

Saline-alkali stress had slight effect on reducing fruit size and fresh weight within the range of 0-90 mM treatments as is shown in Fig. 7 (A, B). Only at the 120 mM level of sodium bicarbonate fruit size and fresh weight were decreased by about 20% as compared to control. Such results could be because of low reduction in water and assimilate accumulation in fruits under the given stress conditions. Fresh weight/size of a fruit could be the product of the total number of cells present in the fruit and their final size, whose enlargement in turn may be affected by the import of water and assimilates. Ho (1996) suggested that fruit enlargement may be regulated by the transport of water and assimilated-carbon into the fruit. Saito et al. (2006, 2009) reported that high EC (8 dS m^{-1}) in hydroponic culture reduced tomato fruit FW and the reduction was attributed to decrease in fruit radius and cell size because of low water influx (Saito et al., 2006). The present result shows that soil EC up to 4.5 dS m^{-1} (0-90 mM) may have little effect on water accumulation in these fruits, therefore fruit size and FW were only slightly affected. In tomato, the principal route of water imported into fruit for growth is regarded via phloem along with sucrose from leaves (Ho et al., 1987; Araki et al., 2004; Plaut et al., 2004). The ratio of phloem water to total water import into fruit was increased at high salinity (Ho at al., 1987), therefore decrease in water uptake in roots may have caused little effect on water transport into fruit. In addition, fruit dry weight accumulation was also unaffected within the whole range of stress treatments (Fig. 8A), therefore assimilates supply was probably unaffected as well.

Fruit dry weight was not affected significantly within the whole range (0-120 mM) of saline-alkali stress treatments (Fig. 8A). Although accumulated dry weight in fruit were higher in 60 and 90 mM treatments than control treatment by 10%, yet this change was non-significant. Nonetheless, fruit dry matter on percent fresh weight basis improved in 60

mM treatment by 20% and in the higher saline-alkali treatments by 25%. These results indicate that saline-alkali stress may improve fruit solid content without reducing fruit fresh weight. Saito et al. (2009) and Gao et al. (1998) while using different carbon isotopes in tomato plants for photosynthesis reported that in saline stress allocation of assimilated carbon is increased to fruit as compared to other sink organs on the plant. The present data regarding FS, FW, and DW, show no significant difference between control and 90 mM treatments and suggest that assimilated carbon may be preferentially allocated to fruit in these treatments, which indirectly support their claim. In addition, high fruit solids in stress conditions as compared to control could also be due to metabolic alteration in fruits (Balibrea et al., 1996). Apart from alterations in the cytoplasmic metabolism of unloaded assimilates, efficient phloem loading in minor veins sieve element of leaf, resulting in high phloem sugar concentration, may also have a role in establishing high solid content of fruit. Sucrose transporter SUT1 is supposed to be the main sucrose loader in the phloem of solanaceous crops (Riesmeier et al., 1993; Burkle et al., 1998; Hackel et al., 2006), and tomato grown under saline-conditions are shown to have up-regulated SUT1 gene expression in leaves (Yin et al., 2010). However, Ruan et al. (1997) showed that when assimilate export from source leaves was increased in two tomato genotypes differing in the sugar content of fruits, the difference in sugar accumulation was not a function of assimilate export from the leaves but the a function of hexose carrier of plasmalemma in fruit sink tissues. Therefore, besides up-regulating the expression of sucrose transporter in leaves, which may increase sugar content of phloem sap, it would be of high interest to know whether salt stress could also increase sucrose unloading efficiency in sink region via the regulation of sugar carriers, i.e. SUT family genes (Shakya and Sturm, 1998), especially when sugar follow the apoplastic route of unloading in fruit, but such study is not reported until now.

3.4.3 Influence on sugar and organic acids accumulation

Glucose and fructose were the major accumulated sugar and their accumulation was regulated in similar pattern under the saline-alkali stress treatments (Fig. 9A, B). 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 mM 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). In short, TSS in fruit of 90 mM treatment was 55% higher than control, while in the other salt treatments TSS was higher but the variance was non significant as compared to control.

The increase in fruit TSS, 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 and Stevens, 1981). Increase in the content of total soluble sugar without reduction in fruit FW in 90 mM treatment, indicates that assimilate accumulation was probably stimulated in these plants. It could be assumed that increase in TSS in stress conditions is not always merely the result of 'condensation effect' as reported (Ho et al., 1987; Sakamoto et al., 1999; Wu and Kubota, 2008), but some intricate changes, like high assimilate transport from leaves and their efficient unloading into fruit 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 sugar in saline stressed tomato plants.

Apart form sugars, organic acids are the major component of fruit SC and flavour,

therefore, the impact of saline-alkali stress on the regulation of organic acids in ripe fruit was also assessed. Citric and malic acids are the major organic acids in ripe tomato fruit in most cultivars (Suarez et al., 2008) and account for about 15% of the fruit SC (Davies and Hobson, 1981). In saline-alkali stress conditions malic and maleic acids of fruits were non-significant in all treatments, but citric acid content, being the major accumulated organic acid, was significantly high in saline-alkali treated plants (Fig. 12A-C). Citric acid content in fruit was increased by 25 - 80% as salt concentration was increased from 60 to 120 mM L^{-1} in comparison to control plants. This 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 and Zeiger, 2006). There are numerous reports underpinning the accumulation of organic acids in tomato fruit as a strategy of the plant to balance the accumulated cations in fruit cells (Davies, 1964; Mitchell et al., 1991a) and 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 can be indirectly assess from the results regarding the escalating accumulation of citric acid in response to increase in the stress levels (Fig. 11B), unlike the TSS accumulation in fruit (Fig. 11A).

In conclusion, this investigation indicates that saline-alkali stress can increase the accumulation of TSS and organic acids in tomato fruit, as is reported in saline stress. High TSS/organic acid content in stressed fruit (90 mM), along with low reduction in fruit dry and fresh weights, reveal such accumulation may not be a function of mere condensation effect. Under other salt stress, fruit SC or TSS show a correlation to rhizospheric EC. Since in this experiment fruit TSS correlated with both EC and pH in saline-alkali conditions (Fig. 13A, B), but there is also a correlation between soil leachate EC and pH, therefore, the role of pH in SC accumulation in tomato fruit was not clear from this experiment. To evaluate the influence of pH on SC, we performed further experiments to investigate SC accumulation in tomato at the similar molar concentration of sodium bicarbonate and sodium chloride salts.

3.5 Summary

Growing of tomato plants in saline conditions is often reported with high solid content in fruit. Saline-alkali soil, unlike saline soil, has high pH besides high EC. Growing of tomato plant in such conditions may influence fruit SC or not is never reported. Therefore, this experiment was performed to investigate the role of saline-alkali stress (0-120 mM) in solid accumulation in tomato fruits. Addition of sodium bicarbonate ($NaHCO_3$) to plants increased pH of soil leachate in 90 and 120 mM stress treatments (above pH 8) in comparison to 0 mM treatment (about pH 6), just two weeks after the salt application. Similarly, soil leachate EC was increased to 5 and 6 $dS \cdot m^{-1}$ at 90 and 120 mM respectively, but in control (0 mM) plants EC gradually decreased from 4 to 1.5 $dS \cdot m^{-1}$. Saline-alkali stress did not decrease fruit size and FW within the range of 0-90 mM, except at 120 mM treatment. Likewise, $NaHCO_3$ application had no effect on fruit DW accumulation. However, the content of fruit dry matter increased significantly from 6.8% at 0 mM treatment to 8.5% at 90 and 120 mM treatments. Total soluble sugar content increased to 3% in 90 mM treatment in comparison to 2% in the control, but starch content remained the same. The increase in TSS was due to significant accumulation of hexose as well as sucrose in ripe fruits. In addition to carbohydrates, saline-alkali stress influenced organic acids accumulation as well. Citric acid, being the major acid, was significantly higher than control at stress level of higher than 30 mM. These results show that saline-alkali stress (0-90 mM) can increase the contents of fruit solid and soluble solid without reducing fruit weight.

Chapter-4

4. Comparative study on the accumulation of solids in tomato fruit under saline-alkali and saline stress conditions during different growing seasons

4.1 Introduction

Tomato, being one of the most important food commodities throughout the world, is a rich source of nutrients and resultantly has received more attention for the improvement of its solid contents (SC). SC of tomato can be improved through various approaches, but the one used widely is subjecting plants to saline or water deficit stress conditions (Mizrahi et al., 1988; Mitchell et al., 1991a, b; Sakamoto et al., 1999; Sato et al., 2006). In the last two decades understanding this issue has been greatly advanced by the physiological as well as molecular level studies (Ho et al., 1987; Gao et al., 1998; Balibrea et al., 1996, 1999, 2003; Plaut et al., 2004; Saito et al., 2008, 2009; Yin et al., 2010). By contrast, no information is available regarding the influence of saline-alkali stress on SC in tomato, in spite of the widespread occurrence of saline-alkali soil throughout the world (Tanji et al., 1996).

Saline and saline-alkali conditions are regarded as two distinct stresses influencing plant growth, organic solutes accumulation, and overall adaptive responses in various species (Yang et al., 2007, 2008a, b, 2009; Liu et al., 2010). In the saline conditions, excessive dissolved salts lower the osmotic potential of rhizospheric water, thus plants have to withstand the adverse affects of both osmotic stress and ions toxicity (Munns, 2002, 2005). By contrast, saline-alkali conditions are suggested to be even more adverse due to the presence of HCO_3^- and/or CO_3^- ions, which can increase the soil pH. Consequently plants have to withstand the adversities of both saline as well as alkali stresses (Yang et al., 2008a, b; Liu et al., 2010). Therefore, most probably, tomato plant in saline-alkali conditions will show different response in regard to SC in fruit.

Presently no literature is available concerning the effect of saline-alkali stress on fruit SC in tomato or other fleshy fruits, however some studies on non-horticultural plants indicate that saline-alkali stress improve SC either in vegetative or reproductive parts. Yang et al. (2007, 2009) studied halophyte and glycophyte plant species growing under saline-alkali and salt stresses. Both species accumulated high soluble sugars and organic acids in leaves under stress conditions, but their contents were much higher in saline-alkali stress in comparison to saline stress. This accumulation was explained as a strategy of the plants to adapt to stress conditions. Alhendawi et al. (1997) have reported such results for some cereal crops, irrigated with water containing sodium bicarbonate. Apart from vegetative parts, bicarbonate rich water can also improve soluble solid contents in wheat grain (Paliwal et al., 1975). These results suggest the role of saline-alkaline stress, in spite of high pH, in increasing SC in various plant parts.

The role of soil pH has been intensively studied in connection to nutrients availability and uptake (see Taiz and Zeiger, 2006). However, if saline-alkali stress conditions could increase SC in fruit or leaves similar to saline conditions, it is possible that rhizospheric pH plays a role in increasing SC. Results in Chapter-3 showed high SC in tomato fruits in $NaHCO_3$ treated plants as compared to control plants. However, this effect could exclusively be due to the high EC of the rhizospheric water or their elevated pH may also have some impact, is not known. We hypothesise that, in saline-alkali conditions, pH along with EC may have a role in increasing SC in tomato fruits. In these experiments, tomato plants were evaluated in summer and winter growing seasons under saline-alkali and saline stress of similar concentration and their mixtures to elucidate the role of soil pH in increasing SC in fruits.

4.2 Materials and methods

4.2.1 Plant materials

Plant materials were the same as described in Chapter-3. Shortly, tomato seeds (*Solanum lycopersicum* L. 'House Momotaro') obtained from Takii (Kyoto, Japan) were soaked for 24 hours on a wet blotting paper and subsequently sowed in soil compost under a glasshouse conditions. Until the unfolding of 4th leaf, seedlings were watered with tap water, and then transplanted into 5-L pots, containing peat-based soil mixture (Soil Mix, Sakata, Japan) and granulated soil (Engei Baido, Kureha, Tokyo) in equal proportion.

Plants were grown in a glasshouse under sunlight and were trained to a single stem by constantly removing axillary shoots. First truss was cut off at anthesis and two fruits were set only on the 2nd truss of the plant, while plant above the 2nd truss was severed at the fruit set of the 2nd truss. First flower of the 2nd truss was removed and the next two flowers of the truss were sprayed once with synthetic plant growth-regulator 'tomato tone' (Ishihara, Japan) for uniform fruit setting. At fruit set of these two fruit the remaining flowers were pinched off. In the winter season, glasshouse was maintained above 15ºC during tight time. The winter experiment was from December 2009 to April 2010, whereas, the summer experiment was from April to July 2010.

4.2.2 Saline and Saline-alkali treatments

For one week after transplanting, plants were watered with tap water and fertilised twice a week with half-strength Otsuka nutrient solution (Osaka, Japan). In these experiments, saline-alkali ($NaHCO_3$ 90 mM), saline (NaCl 90 mM), and mix treatments of the two salts, i.e. $NaHCO_3$ 60 mM + NaCl 30 mM (Mix-1) and $NaHCO_3$ 30 mM + NaCl 60 mM (Mix-2) were given to plants for the whole growing period in both seasons.

For these treatments, sodium bicarbonate ($NaHCO_3$) or sodium chloride (NaCl) salt

solutions were mixed in 1L tap water. In all salt treatments, every plant was given 1L solution twice a week at 2 to 3 days interval. These plants were fertilised with half-strength Otsuka nutrient solution two times weekly, usually one day prior to salt treatment.

Control plants were fertilised twice a week with half-strength Otsuka nutrient solution. These plants were irrigated with tap water when required, assessed by visual observation of the soil. However, every time, equal volume of water (1L) was applied to each plant to avoid variation.

4.2.3 Recording soil leachate pH/EC and fruit sampling

In all treatments, after applying salt or nutrient solutions to the plants, the leached-out solutions were collected for each plant. pH and EC of the soil leachate was recorded once a week when $NaHCO_3$ or NaCl solutions were applied in stressed plants or nutrient solution were applied in control plants. About 30 ml soil leachate was sampled in 50 ml tube and their EC was measured with a portable EC meter (CM-14P, TOA Electronics Ltd., Japan) and pH with a pH meter (210, Beckman Instruments, Inc., USA).

At turning stage of fruit growth, 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, 5 to 6 days after turning stage, both of the two fruits on the truss were harvested for analysis at dusk. Fruit were cut into four pieces at vertical direction, and two alternate pieces were sampled and stored at –18ºC. These samples were freeze-dried and analysed for soluble sugars and organic acids.

4.2.4 Carbohydrates and organic acids analysis

Carbohydrates and organic extraction were the same as reported in Chapter-2. About 100 mg samples were extracted with 80% ethanol; 2 hours for sugar and 30 minutes for organic acids extraction, at 85ºC. The extracts were filtered, dried in a rotary evaporator, and

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 elutes 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 system (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) solution 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) set at 210 nm. Phosphoric acid (0.1 %) was used as isocratic flow mobile phase with flow rate of 0.8 ml·min^{-1}.

Starch was extracted through boiling alcohol insoluble fraction in water and the glucose contents were determined enzymatically using amyloglucosidase (EC 3.2.1.3, 142 U mg^{-1}, 10113, Sigma) and Glucose assay Kit (Glucose CII, Wako), and starch contents were estimated by multiplying the glucose content by 0.9.

For these experiments, plants were arranged according to randomised complete block design in the glasshouse, each treatment having 10-15 replications. Data was analysed according to one-way analysis of variance (ANOVA) and means were compared by using Student-Newman-Keul's test.

4.3 Results

4.3.1 Soil leachate pH and EC

pH of the soil leachate reveals very contrast results for the saline-alkali and saline treatments during winter as well as summer growing seasons (Fig. 14A, C). pH in saline-alkali treatments after 4 weeks was the highest among all the treatments (8.5), but did not increase further until the end of the growing periods. Unlike in the saline-alkali treatment, pH in the saline treatments was as low as control treatment, within the range of 6 to 7 throughout the experimental period during both seasons. Moreover, pH in the Mix-1 and Mix-2 treatments was between that of the saline-alkali treatments ($NaHCO_3$ 90 mM) and saline treatments (NaCl 90 mM), and followed the similar pattern of change. Mean data also shows saline-alkaline treatment had the highest pH and saline treatment was equivalent to control (Fig. 14E).

The result of soil leachate EC in saline-alkali and saline treatments was in opposite to their pH levels. In 90 mM NaCl treated plants, EC of the soil leachate increased gradually from 6 to 15 and 19 $dS \cdot m^{-1}$ in winter and summer crop respectively (Fig. 14B, D). In plants treated with 90 mM $NaHCO_3$, EC of the soil leachate remained within the range of 4 to 6 $dS \cdot m^{-1}$ in the winter crop and 5 to 8 $dS \cdot m^{-1}$ in the summer crop throughout the growing season. In Mix-2 treatments, EC was close to NaCl treatment in both experiments, whereas Mix-1 was much lower. As observed in Chapter-3, soil leachate EC in control treatments decreased gradually from 4 to 1.5 $dS \cdot m^{-1}$ in the whole growing period. Mean value for EC was about 3 $dS \cdot m^{-1}$ in control treatment, 4-6 and 10-14 $dS \cdot m^{-1}$ in saline-alkali and saline treatments in both seasons (Fig. 14F).

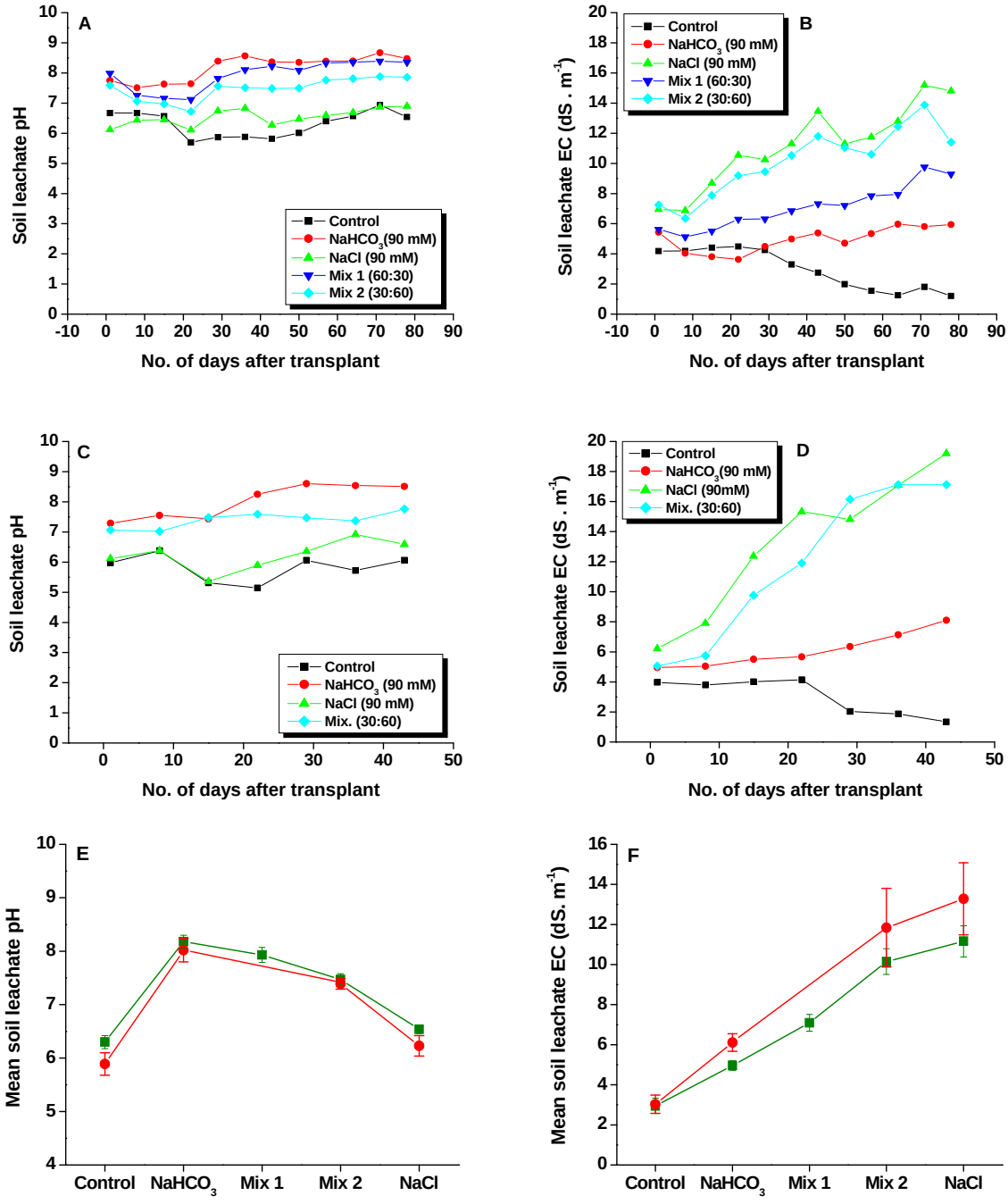


Fig. 14. Effect of sodium bicarbonate, sodium chloride and mix–salts treatment on soil leachate pH and EC. A and B show data regarding pH and EC in winter tomato, C and D show data regarding summer tomato. E and F show mean data for pH and EC respectively, where green colour shows data for winter crop and red colour shows data for summer crops. Each point represents mean value of n= 4. Mix-1 salt treatment was not included in the summer season experiment.

Mix-1 is mixture of 60 mM $NaHCO_3$ + 30 mM NaCl.

4.3.2 Fruit fresh and dry weight accumulation

In all stress treatments, fruit cross sectional area (size) and fresh weight (FW) were reduced significantly, in both seasons, in comparison to control treatment (Fig. 15A, B). In winter crop, fruit size dropped significantly from 60 cm^2 to about 30 cm^2 in saline-alkali treatments. The size of the fruit was similar in all stress treatments without any significant difference. In summer crop, control plants fruit had a mean size of 50 cm^2, which was lowered to about 35 cm^2 in all stressed plants. Exactly the same pattern of change, as in size, was observed for fruit fresh weight (Fig. 15B). FW decreased from 300 g and 200 g, in winter and summer crop, respectively, to about 120 g in saline-alkali as well as saline and mix-salt treatments.

Saline-alkali and the rest of stress treatments had similar fruit dry weight (DW), but their weight was significantly lower than those of the control in both seasons (Fig. 16A). Fruits from control plants had DW of 23 g (in winter) and 15 g (in summer), while DW in stressed plants was reduced to 15 g (winter) and 10 g (summer) in both experiments. The differences in the dry weight of fruits in all stress treatments were non-significant. Nevertheless, fruit dry matter percentage (DM), unlike to control plants, was remarkably increased by all the stress treatments (Fig. 16B). In winter fruit, DM in control plant was 7.5%, which rose to 14% in saline-alkaline and saline stress, whereas in mix-salt treatments it remained 12.5%. In summer crop, fruit DM increased from 6.9% in control to 8% in saline-alkali and 9.6% in saline treatments. Differences in DM content in stress treatments were non-significant in both seasons.

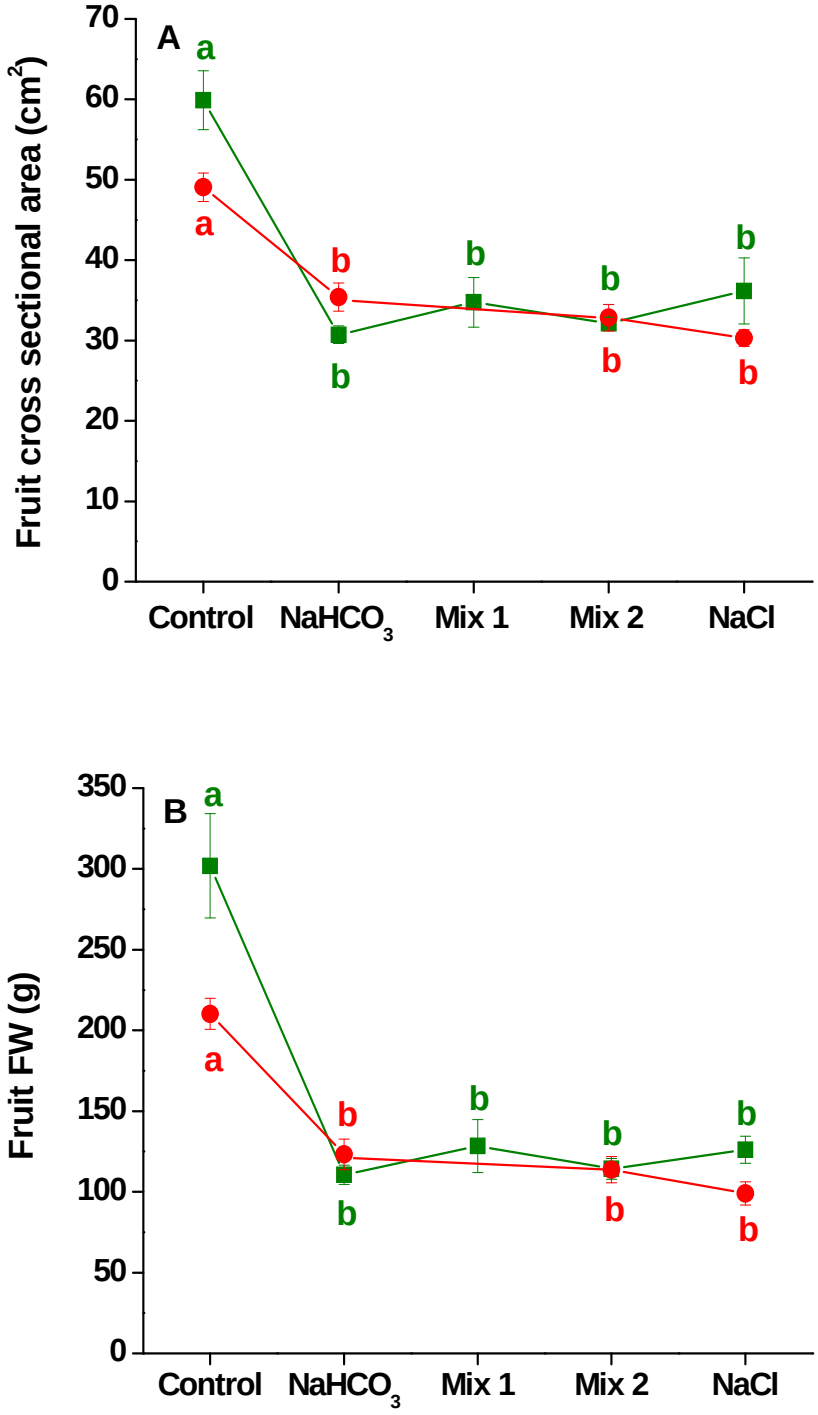


Fig. 15. Effect of sodium bicarbonate, sodium chloride and mix–salts treatment on fruit cross sectional area (A) and FW (B). Green and red colours show data for winter and summer crops, respectively. Error bars indicate ± SE. No. of replicates are n= 4-8 (winter) and n= 7-10 (summer). Different letters between treatments indicate that differences are significant at $P<0.01$. Mix-1 salt treatment was not included in the summer Exp. Mix-1 is mixture of 60 mM $NaHCO_3$ + 30 mM NaCl.
Mix-2 is mixture of 30 mM $NaHCO_3$ + 60 mM NaCl.

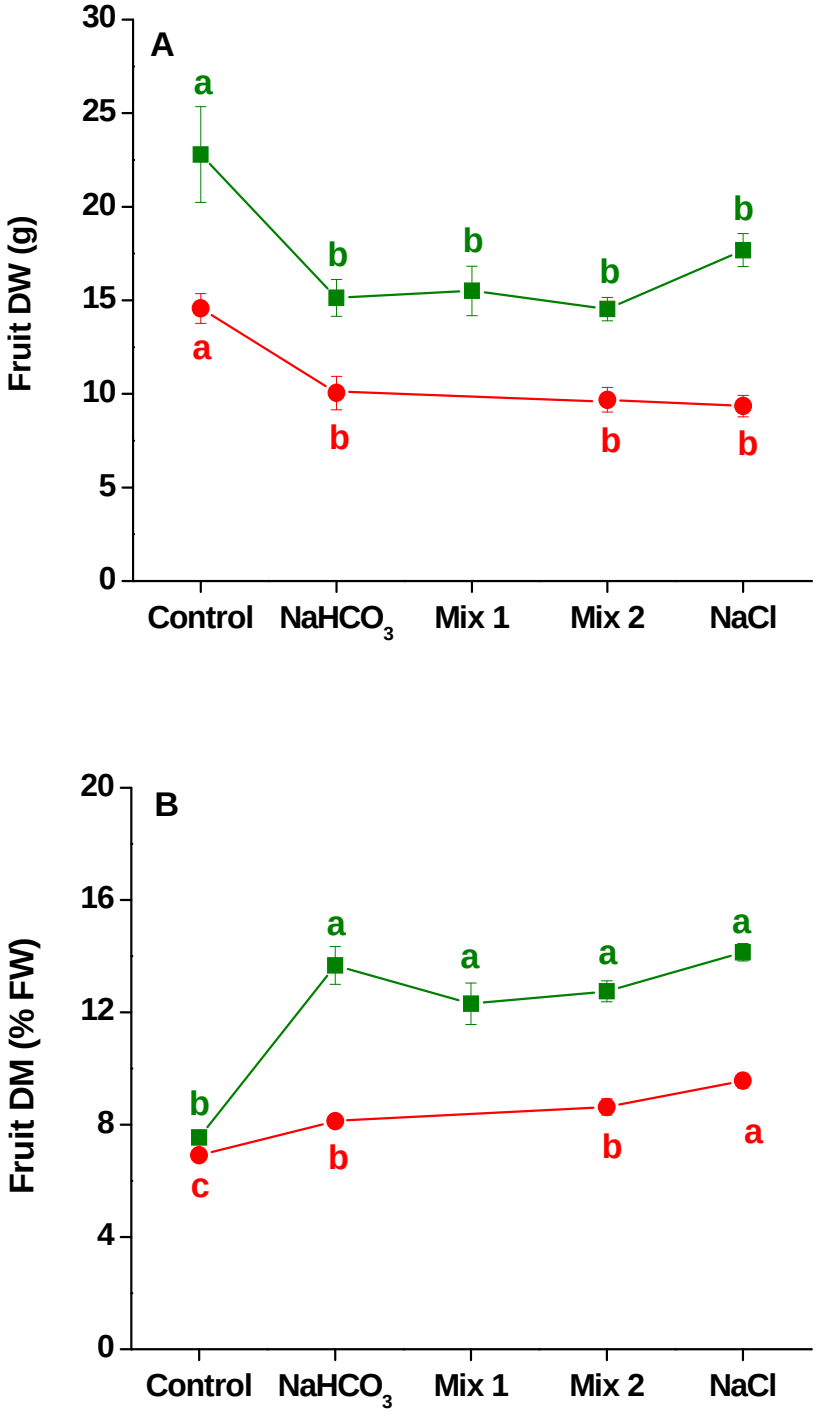


Fig. 16. Effect of sodium bicarbonate, sodium chloride and mix-salts treatment on fruit dry weight (A) and % DM (B). Green and red colours show data for winter and summer crops, respectively. Error bars indicate ± SE. Where error bars are not visible, they are smaller than the symbol. No. of replicates are n= 4-8 (winter) and n= 7-10 (summer). Different letters between treatments indicate that differences are significant at $P<0.01$. $P<0.05$ for DW in winter. Mix-1 salt treatment was not included in summer Exp.

Mix-1 is mixture of 60 mM $NaHCO_3$ + 30 mM NaCl.

Mix-2 is mixture of 30 mM $NaHCO_3$ + 60 mM NaCl.

4.3.3 Soluble sugar and organic acid accumulation

Fruit glucose and fructose (hexose) content revealed somewhat similar results in both seasons. Winter crop fruit registered significant increase in hexose accumulation in saline-alkaline treatment (2.3% FW) as compared to control plants (1.4% FW) (Fig. 17A, B). The hexose content of summer crop was also high in saline-alkali treated plants (1.5% FW) than control plants (0.9% FW). In both seasons, glucose and fructose concentration in fruits of saline and mixed-salt stress treatments were statistically similar to saline-alkali stress.

Likewise, sucrose content also increased in stress treatments. In winter crop, its content was increased by all treatments similarly, in comparison to the control. Whereas in summer crop the sucrose content of saline-alkali treatment was higher than control, but it was even further accumulated in saline treatment (Fig. 18A). The accumulation of sucrose in stressed conditions was not only significant on fresh weight basis, but also on dry weight basis (Fig. 18B).

Figures 19A, B show the contents of starch and total soluble sugar (TSS). Starch content was relatively higher in stress treatments than control in winter crop, whereas only saline stress increased starch content in summer crop. TSS content was 3% in control and 5% in saline-alkali and Mix-2 treatments. TSS in saline treatments and Mix-1 were about 4.5% on fresh weight basis. In summer crop, TSS in control fruit was 1.8%, which increased up to 3.2% in saline-alkali, 3% and 3.6% in saline and Mix-2 treatments respectively.

Malic and maleic acid concentration was less than 1 $mg \cdot g^{-1}$ FW, but all salt treatments increased it significantly (Fig. 20A, B). The maximum amount of malic acid accumulated in saline-alkali treatments in winter experiment, while in summer crop the

effect was non conspicuous among treatments. Maleic acid was only high in Mix-2 treatment in summer crop.

Citric acid was the major accumulated organic acid in both seasons, but its content was much higher in winter crop (Fig. 21). Fruits in the saline-alkali treatment had the highest content of citric acid (8 $mg \cdot g^{-1}$ FW), followed by saline (7.5 $mg \cdot g^{-1}$ FW), and the mixtures of both salts (5 to 6 $mg \cdot g^{-1}$ FW). The content of citric acid in control plants was as low as 3 $mg \cdot g^{-1}$ FW. Summer crop had comparatively low citric acid content in stress treatments. Saline-alkali treatment had similar content as control treatment, but higher than saline treatment.

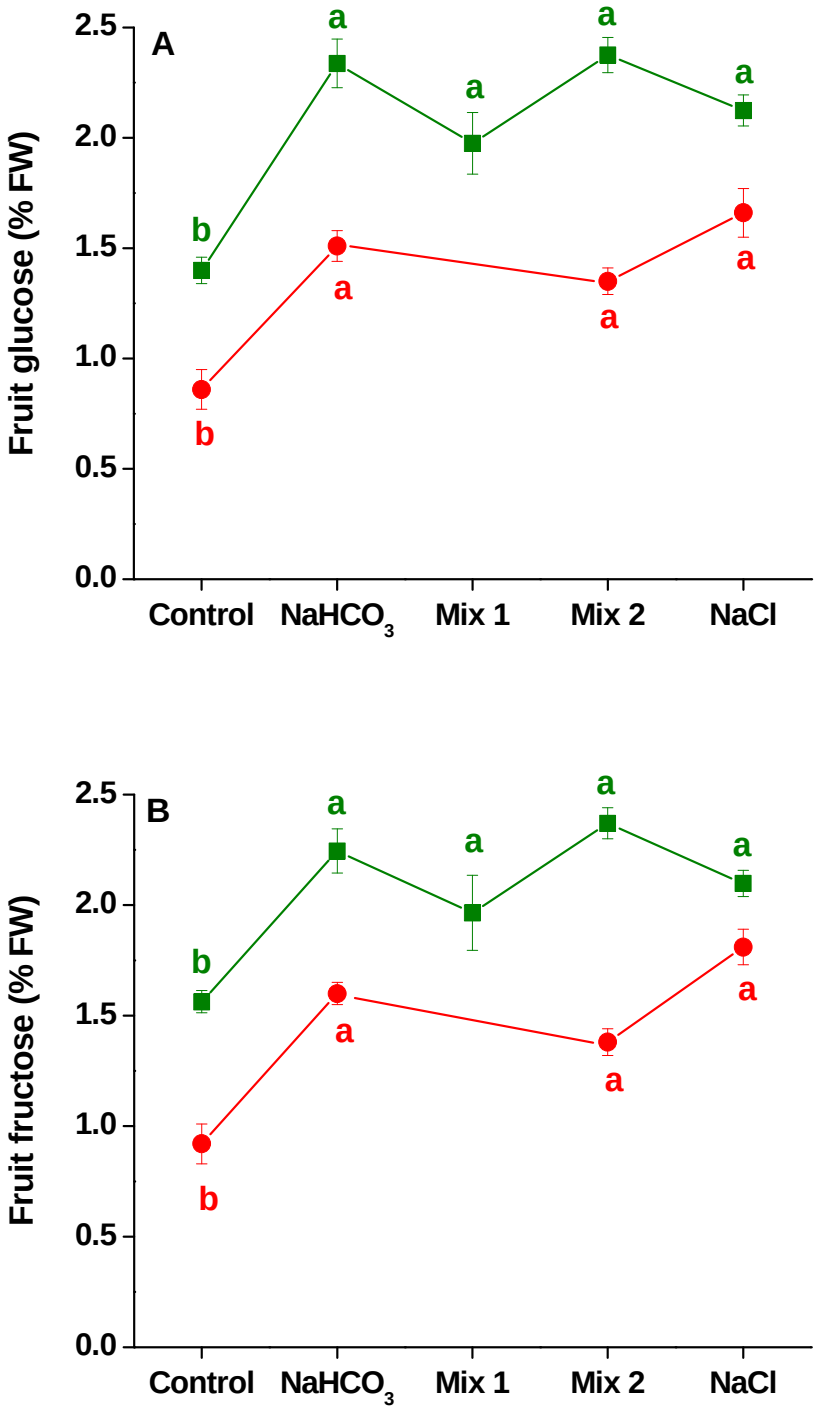


Fig. 17. Effect of sodium bicarbonate, sodium chloride and mix-salts treatment on glucose (A) and fructose (B) content of fruit. Green and red colours show data for winter and summer crops, respectively. Error bars indicate ± SE. No. of replicates are n= 4-8 (winter) and n= 7-10 (summer). Different letters between treatments indicate that differences are significant at $P<0.01$. $P<0.05$ for fructose content in winter. Mix-1 salt treatment was not included in summer Exp.

Mix-1 is mixture of 60 mM $NaHCO_3$ + 30 mM NaCl.

Mix-2 is mixture of 30 mM $NaHCO_3$ + 60 mM NaCl.

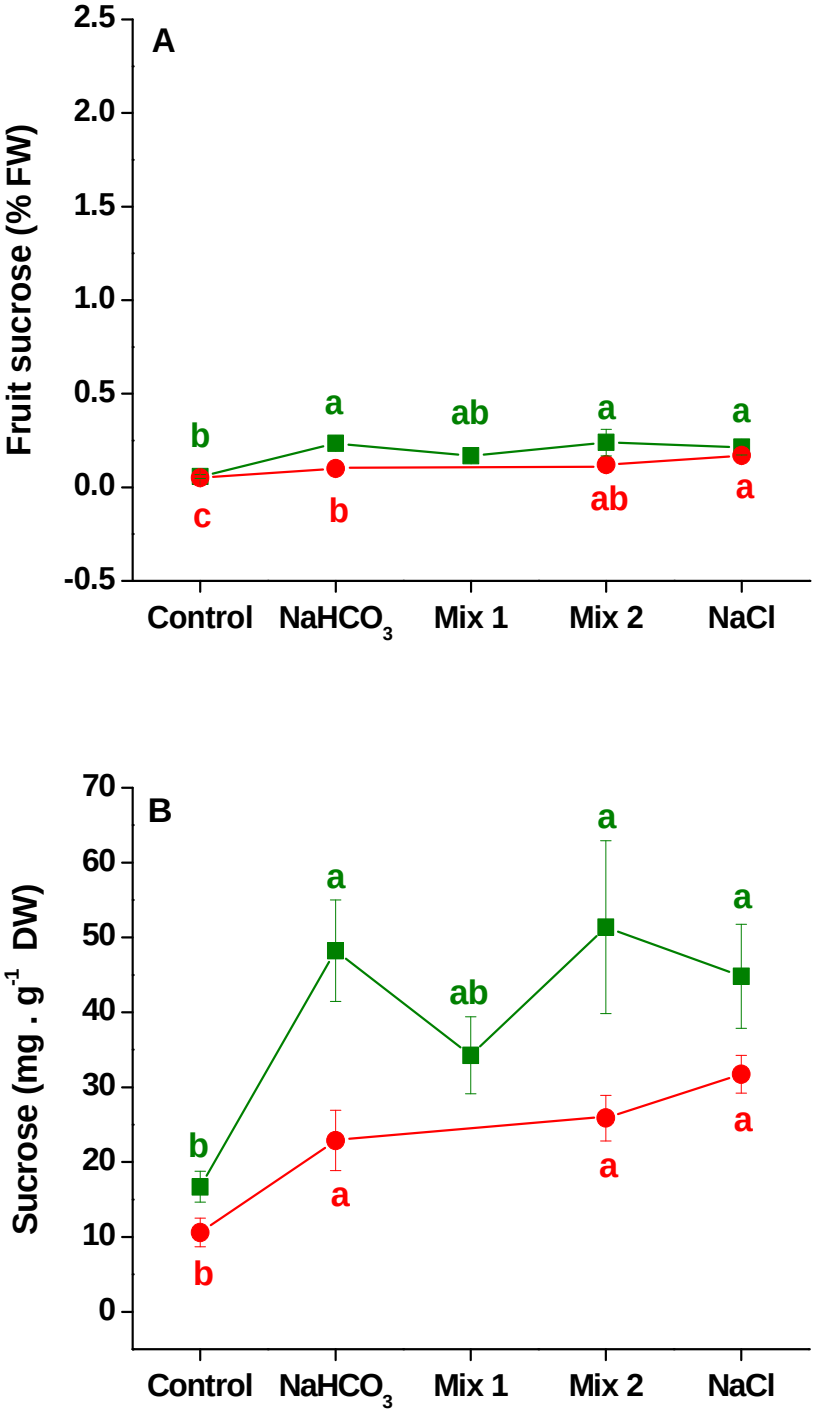


Fig. 18. Effect of sodium bicarbonate, sodium chloride and mix-salts treatment on sucrose content (FW basis) (A) and (DW basis) (B) of fruit. Green and red colours show data for winter and summer crops, respectively. Error bars indicate ± SE. Where error bars are not visible, they are smaller than the symbol. No. of replicates are n= 4-8 (winter) and n= 7-10 (summer). Different letters between treatments indicate that differences are significant at $P<0.01$. $P<0.05$ for sucrose content in winter. Mix-1 salt treatment was not included in summer Exp. Mix-1 is mixture of 60 mM $NaHCO_3$ + 30 mM NaCl.
Mix-2 is mixture of 30 mM $NaHCO_3$ + 60 mM NaCl.

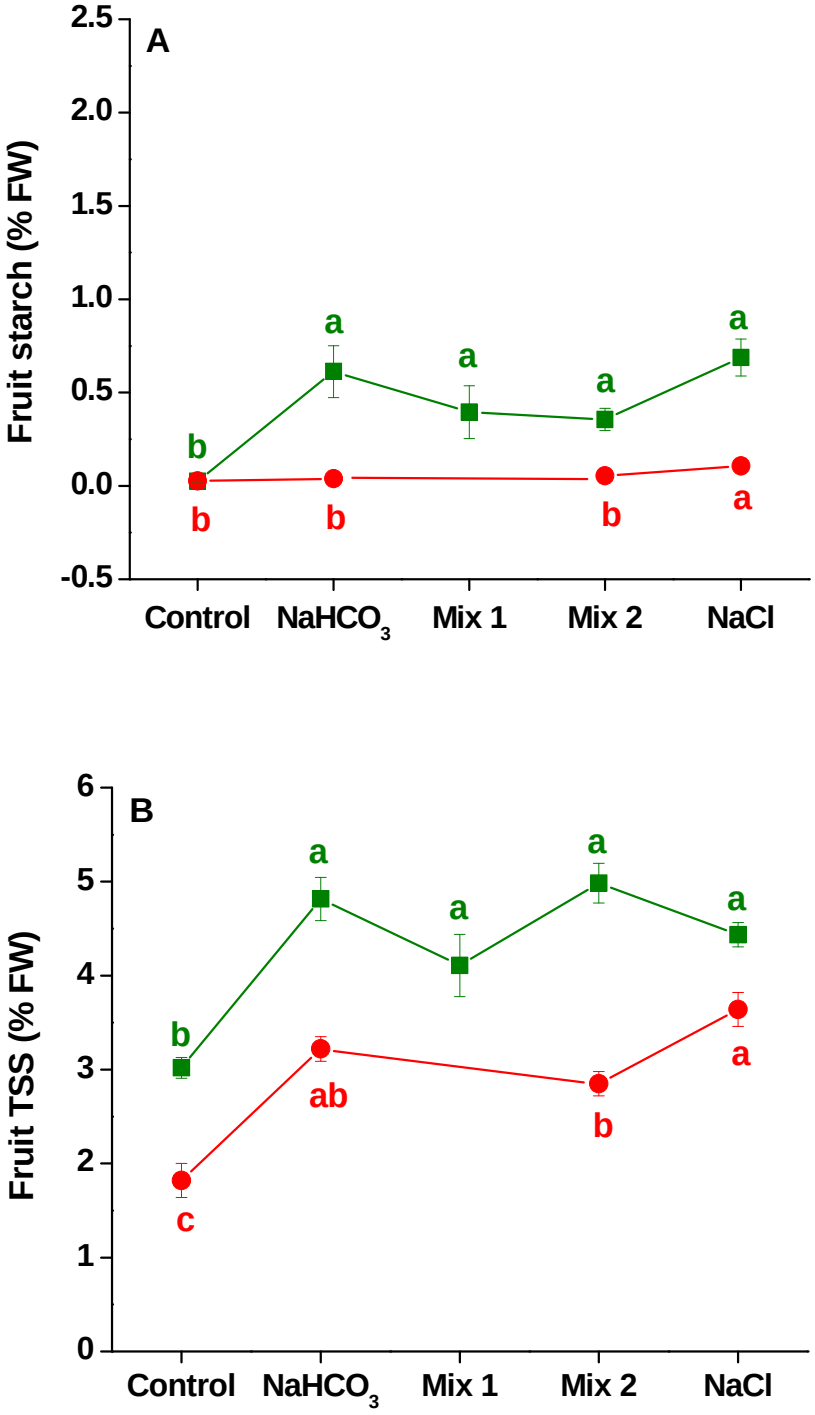


Fig. 19. Effect of sodium bicarbonate, sodium chloride and mix-salts treatment on starch (A) and total soluble sugar (B) content of fruit. Green and red colours show data for winter and summer crops, respectively. Error bars indicate ± SE. Where error bars are not visible, they are smaller than the symbol. No. of replicates are n= 4-8 (winter) and n= 7-10 (summer). Different letters between treatments indicate that differences are significant at $P<0.01$. Mix-1 salt treatment was not included in summer Exp. Mix-1 is mixture of 60 mM $NaHCO_3$ + 30 mM NaCl. Mix-2 is mixture of 30 mM $NaHCO_3$ + 60 mM NaCl.

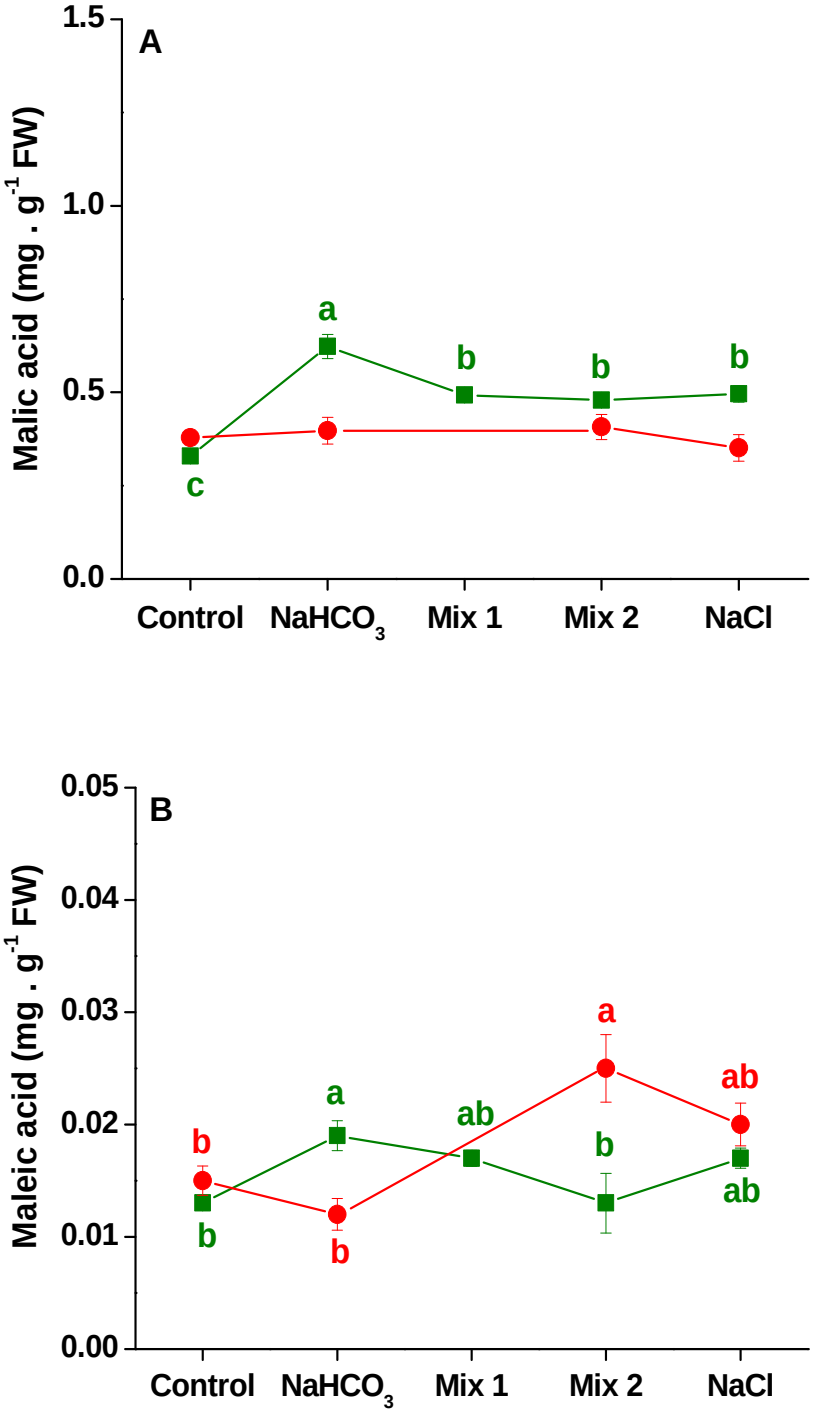


Fig. 20. Effect of sodium bicarbonate, sodium chloride and mix-salts treatment on malic (A) and maleic acids (B) content of fruit. Green and red colours show data for winter and summer crops, respectively. Error bars indicate ± SE. Where error bars are not visible, they are smaller than the symbol. No. of replicates are n= 4-8 (winter) and n= 7-10 (summer). Different letters between treatments indicate that differences are significant at $P<0.05$ for malic and maleic acid content in both season. Mix-1 salt treatment was not included in summer Exp. Mix-1 is mixture of 60 mM $NaHCO_3$ + 30 mM NaCl. Mix-2 is mixture of 30 mM $NaHCO_3$ + 60 mM NaCl.

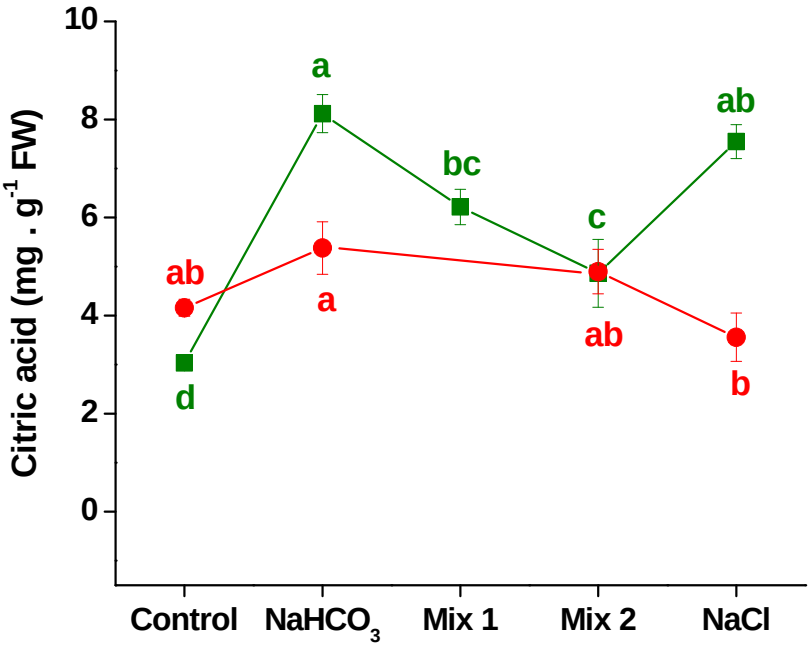


Fig. 21. Effect of sodium bicarbonate, sodium chloride and mix-salts treatment on citric acid content of fruit. Green and red colours show data for winter and summer crops, respectively. Error bars indicate ± SE. Where error bars are not visible, they are smaller than the symbol. No. of replicates are n= 4-8 (winter) and n= 7-10 (summer). Different letters between treatments indicate that differences are significant at $P<0.01$ in winter and at $P<0.05$ in summer crop. Mix-1 salt treatment was not included in summer Exp.

Mix-1 is mixture of 60 mM $NaHCO_3$ + 30 mM NaCl.

Mix-2 is mixture of 30 mM $NaHCO_3$ + 60 mM NaCl.

4.4 Discussion

4.4.1 Influence of $NaHCO_3$ and NaCl salts on the rhizospheric EC/pH regulation

Experimental data, in both growing seasons, revealed that addition of sodium bicarbonate salt can increase pH and EC of the soil leachate (Fig. 14A, C, E). Hence stress can be imparted to plants soil in nature. It seems noteworthy that in saline-alkali stress, though equal to saline stress in salt concentration, EC of the soil leachate was not as high as in saline conditions (Fig. 14 B, D, F). This variance may be because of the different chemical nature of Cl^-, Na^+ and HCO_3^- ions influencing EC of root-zone. The presence of both Na^+ and Cl^- ions in soil may influence EC of the leachate different than the combine effect of Na^+ and HCO_3^-, as is also reflected in the mix-salt treatments. In saline-alkali conditions, pH was higher than 8, whereas in saline condition pH was similar to control treatments (6-7). Based on such differences, it was argued that salt-alkali and salt stresses impose distinct kinds of stresses for plant growth (Yang et al., 2008a). It appears that tomato SC would also be influenced differently in these conditions. If saline-alkali stress can improve the solid content of fruits as reported in saline stress, it would be interesting to assess the role of rhizospheric pH also.

Saline-alkali, saline, and mix-salt treatments affected fruit cross sectional area, FW, and DW in similar fashion in both seasons (Fig. 15, 16). Fruit FW was decreased by 60% in winter and 40% in summer crop in saline-alkali stress treatment, whereas fruit DW was lowered by 30% in comparison to control. This reduction in weight in saline-alkali and other stress treatments was similar in extent. Thus, all these stress treatments seemingly restricted the accumulation of assimilated carbon (Saito et al., 2009) as well as water (Ho, et al., 1987; Plaut et al., 2004), which are reported the limiting factors to affect fruit cross sectional area and FW (Bolarin et al., 2001). Gao et al. (1998) observed significant reduction in photosynthetic rate in tomato leaves grown in 100 mM NaCl, owing to change

in stomatal conductance and transpiration. Likewise, Balibrea et al. (2000) observed significant reduction in the photosynthetic activity of individual leaf as well as total photosynthesis per plant at 0-100 mM NaCl stress in salt-tolerant and sensitive tomato genotypes. The present results showing 30% reduction in fruit dry weight could be attributed to decreased photo-assimilation. Nonetheless, the content of dry matter of the fruit (% DM), in all stress treatments, was higher than control in winter (65-85%) and summer (20-40%) crops (Fig. 16B). Since the reduction of fruit total solid accumulation was less than fresh weight (water) accumulation (Ehret and Ho, 1986b), increase in the content of dry matter was most likely as is often reported for saline stress conditions (Ehret and Ho, 1986b; Adams, 1991; Mitchell et al., 1991a).

4.4.2 Does pH influence fruit water accumulation?

Present data also indicate that high pH observed in saline-alkali treatment (Fig. 14E) did influence water accumulation in fruit. Frequently observed reduction in plant/fruit fresh weight in salt stress conditions is usually attributed to low osmotic potential of soil water at high EC. Soil leachate EC in saline-alkali treatment was more than two times lower than saline and Mix-2 treatments (Fig. 14F), but there was no apparent difference in the FW of their respective fruits (Fig. 15B). Moreover, if the content of fruit dry matter on FW basis is increased in proportion to EC of root-zone, as reported (Li et al., 2001), fruit from lower EC treatments (saline-alkali) must have lower DM content accordingly, which is not the case in these results (Fig. 16B). Water uptake by tomato plant (Ehret and Ho, 1986b; Reina-Sanchez et al., 2005) and relative water content of fruits (Li et al., 2001) were proposed in inverse relationship to root zone EC; therefore it appears that in saline-alkali conditions, soil pH may also have influenced plant water uptake and thereby fruit water content. This possibility can stem from the result of similar decrease in FW and fruit cross sectional area in all stress treatments, in spite of contrast variance in rhizospheric EC of

these treatments, during different growing seasons.

4.4.3 Sugar accumulation in saline-alkali/saline stress conditions

Apart from the fruit capacity to import assimilates, the partitioning of those assimilates into soluble and insoluble carbohydrates inside cells could be a defining feature of quality. In ripe fresh tomato fruit, hexose accounts for about 50% of the dry matter (Davies and Hobson, 1981), wherein an increase is associated to edible quality improvement (Saito et al., 2006). Saline stress conditions appear to influence this trait of fruit either through decreasing water accumulation of fruits (Ho et al., 1987; Mitchells et al., 1991b; Sakamoto et al., 1999), or promoting carbon translocation to fruits (Gao et al., 1998; Saito et al., 2009).

The present results showed fruit glucose content increased significantly in response to saline-alkali (winter 70%, summer 75%) as well as saline (winter 50%, summer 95%) treatments (Fig. 17A). Fruit fructose content was also higher in winter crop (45%) and summer crop (75%) in saline-alkali as well as saline (winter 35% and summer 95%) and mix-salt treatments (Fig. 17B). Such accumulation of hexose is specific feature of plants under stress conditions and is reported frequently. In early stage of fruit development, fruit accumulates carbohydrates partly as starch (Ho et al., 1983), whose synthesis and accumulation was suggested to increase under salt stress (Gao et al., 1998; Yin et al., 2010). At ripening stage these reserves are hydrolysed into glucose, thus adding to the total hexose content of fruits (Dinar and Stevens, 1981). Therefore, it is proposed that reduction in water import into fruit (Ho et al., 1987; Sakamoto et al., 1999), and the accumulation of starch in early developing tomato fruits (Schaffer and Petreikov, 1997; Yelle et al., 1988), could be a likely explanation for high hexose content in salt stressed plants. It has been proposed that under saline stress, hexose accumulation in tomato fruits is increased as an adapting response to low water potential by regulating fruit osmotic potential (Mitchell et

al., 1991a, b; Plaut, et al., 2004).

4.4.4 Accumulation of sucrose in saline-alkali/saline conditions different than hexose

It is interesting that in all stress treatments accumulation of fruit sucrose was proportionally much higher than hexose, in spite of its low content (Fig. 18A). In saline-alkali treatment sucrose accumulation was 300% and in saline plants was 270% higher, in winter crop, than control. In summer crop its accumulation was higher by 120% or 260% in saline-alkali and saline treatments, respectively. The increase in fruit sucrose content among all stress treatments was non-significant in winter crop. Some reports show salinity does not influence final sucrose content in ripe fruits (Gao et al., 1998; Sato et al., 2006; Lu et al., 2010). However, other reports have shown significant accumulation of sucrose as in the present study (Balibrea, et al., 1996, 1999; Saito et al., 2008; Yin et al., 2010). Since accumulation of sucrose in all stress treatments was increased on fruit dry weight basis as well (Fig. 18B), therefore the condensation of fruit constituents may not be an acceptable explanation in these experiments. Low sucrolytic activity or re-synthesis of sucrose inside the cell cytosol could be the reason for such high sucrose in these stress conditions.

Sucrose unloading into tomato fruit was proposed via apoplastic pathway during the late developmental stage of fruit (Ruan and Patrick, 1995). Since cell-wall invertase activity remains very high in the late developmental stage of tomato (Yelle, et al., 1988; Sato et al., 1993), therefore hydrolysis of sucrose by well wall invertase is proposed to play a principal role for sucrose unloading via apoplastic pathway. Balibrea et al. (1996) had proposed decrease in the hydrolysis of sucrose via acid invertase as a reason for sucrose accumulation in fruits. They speculated inactivation of cell-wall invertase due to a change in the apoplastic pH and/or effect of Na^+ accumulation. In tomato, acid invertase activity is pH specific with optimum activity around pH 4.5 (Pressey, 1994), therefore, any change in

pH of fruit apoplast due to accumulation of Na^+ may affect its activity.

Balibrea et al. (1999) reported increase in the accumulation sucrose in the fruits growing under saline conditions, owing to acidification of apoplast due to Na^+ accumulation and low activity of insoluble acid invertase. Chookhampaeng et al. (2008) reported an increase in the amount of sucrose with concomitant increase in the activity of sucrose phosphate synthase (SPS) in tomato fruit under salinity. While sucrose accumulation in sucrose accumulating tomato species *L. chmielewskii* was attributed to low insoluble acid invertase activity and high sucrose phosphate synthase activity (Dali et al., 1992). Since insoluble acid invertase is the major enzyme for sucrose hydrolysis in the late stage of fruit development, while SPS is a key enzyme for sucrose re-synthesis in cell cytosol (Nguyen-Quoc and Foyer, 2001). Therefore, it can be assumed that high sucrose accumulation in the present study could be the result of such metabolic alterations.

4.4.5 Effect of saline/alkali stress on fruit organic acids

Organic acids are the second highest component of the tomato solid and determinant of quality (Davies and Hobson, 1981), therefore, their accumulation along with sugar will have a profound effect on the fruit edible quality as well.

Under all stress treatments, citric and malic acids were the major accumulated organic acids, whereas maleic acid was in trace amount (Fig. 20A, B). Citric acid content was increased by 170% in saline-alkali treatment (Fig. 21). Accumulation of organic acids under salt stress conditions is proposed to be a strategy of plants to withstand unfavourable circumstances (Guo et al., 2010), arising from the excessive uptake of salt cations in roots (Mitchell et al., 1991a). Plant cytosolic pH is weakly alkaline (~7.5), while vacuolar pH is acidic (~ 5.5) (Taiz and Zeiger, 2006). Since most inter- and intra-cellular enzymes are sensitive to pH, plant cells may regulate their pH within a narrow range. Therefore, plant

may maintain the level of pH, possibly by accumulating organic metabolites or inorganic ions. In barley seedlings, addition of NaCl caused alkalinisation of the vacuole of roots (Martinez and Lauchli 1993; Katsuhara et al., 1996), which was associated with Na^+ accumulation (Martinez and Lauchli 1993). Such accumulation of cations in plant cell may change the intra-cellular and vacuolar cation to anion ratio. Since organic acids are sequestered in the vacuole of fruit parenchyma, it is probable those fruit cells accumulate and/or synthesise organic acids in access, as an anion salt, to maintain vacuolar cation to anion ratio and pH. In saline conditions, Cl^- is the major accumulated anion in plant cell and influence the cation to anion ratio (Mitchell et al., 1991b; Guo et al., 2010). In contrary, in saline-alkali conditions HCO_3^-/ CO_3^{2-} is the anion component of the salt, therefore, plant under such conditions may have some other strategy to balance the ionic ratio and maintain cell pH.

It appears from the present data that saline-alkali stress increases organic acids accumulation in particular. The data in Chapter-3 also indicated that citric acid accumulated as sodium bicarbonate salt concentration increased in the medium (Fig. 11B). A possible explanation for this increase could be the presence of bicarbonate ions in soil, plant shoot, and roots in saline-alkali conditions. Scores of report using carbon isotopes suggested that plant roots absorb HCO_3^- and is readily incorporated into organic compounds and acids (Arteca and Poovaiah, 1982; Vapaavuori and Pelkonen, 1985; Bialczyk and lechowski, 1992; Vuorinen et al., 1992), particularly into malic acid (Arteca and Poovaiah, 1982; Bialczyk and lechowski, 1992), in the presence of phosphoenolpyruvate carboxylase. Malic acid is translocated from roots to shoot, where it can either be decarboxylated and the carbon is re-fixed by rubisco (Hibbard and Quick, 2002), or it is transported into fruits along with sugar (Walker and Ho, 1977). Davies and Maw (1972) reported that malic acid, the most active metabolite of tricarboxylic acid cycle,

is actively converted into citric acid in the ripe tomato fruit. The absorption and fixation of bicarbonate ions into malic acid and its active conversion into citric acid could also be expected in saline-alkali treatments.

In the present work, all organic acids, particularly citric acid was higher in saline-alkali treatment than saline treatment or mix-salt treatments. This would not be high speculation to assume that the presence of HCO_3^- in saline-alkali treatment could be the reason for high organic acid in the fruit. Though in mix-salt treatments citric acid was significantly lower than saline-alkali treatment, it appears that the presence of extra Cl^-, in both conditions, may have sufficed the anion content to maintain the intra-cellular cation to anion ratio.

The main objective of this study was to address the question; whether soil pH also has a role in tomato SC accumulation, as is reported for soil EC. McEnroe and Coulter (1964) has reported an increase in sugar content and sugar yield of sugar beet crop when soil pH increased from below 6.0 to above 7.0. In the present study the contents of DM and TSS at saline-alkali treatment (pH 8) was higher than control (pH 6.5), but equal to saline treatment (pH 6-7). Since in saline-alkali treatment, having 2-3 times low soil EC but 2 units high pH than saline treatment (Fig.14 E, F), reduction in fruit fresh weight or increase in DM content was equivalent to saline treatment (Fig. 15B). This suggests changes in fruit weight or DM content is related not only to high rhizospheric EC (Li et al., 2001) but also pH. Therefore, it is proposed that plant rhizospheric pH improve tomato fruit SC. In a halophyte grass, Li et al. (2010) observed high carbohydrates accumulation in leaves by salinity as well as by high pH. But interestingly, carbohydrates accumulation was not affected by high soil pH in the absence of salinity. These observations suggested that carbohydrates accumulation in tomato is probably a mutual affect of soil salinity (EC) and soil pH.

To summarise, these results show that saline-alkali stress can increase SC in tomato fruit to the same extent as in saline stress. In saline-alkali stress, increase in TSS of fruits could be similar in extent to saline stress but increase in organic acids content could be higher. In saline-alkali stress, soil leachate was two times lower and pH was two units higher than in saline stress but increase in dry matter content was similar in extent. These observations suggest, soil pH play a role to increase solid content in saline-alkali condition.

4.5 Summary

Increasing number of evidence suggest the role of high rhizospheric EC in enhancing SC of tomato fruit. Data in previous chapter showed that saline-alkali stress, retaining high pH in addition to high EC in the medium, can improve SC of tomato fruits. To elucidate the role of soil pH in improving the SC of fruits, tomato plants were grown in saline-alkali ($NaHCO_3$), saline (NaCl), and mix-salt ($NaHCO_3$+NaCl) conditions in two different seasons. Results indicated that saline-alkali treatment increased soil-leachate pH (8.5), while pH was similar in saline and control treatments (pH 6). On the contrary, soil-leachate EC was two times lower (5-6 $dS \cdot m^{-1}$) in saline-alkali treatment than saline treatment (11-13 $dS \cdot m^{-1}$). Fruit fresh weight and dry weight decreased in all treatments to similar extent. Dry matter content of fruits increased significantly from 7 to 14% and TSS from 3 to 5% in all treatments. The content of sucrose on a fresh weight as well as dry weight basis increased in all stress treatments. Organic acids accumulated in stressed fruit higher than control, but their content was higher particularly in saline-alkali treatment. Stress treatments increased citric acid content but the maximum content (8 $mg \cdot g^{-1}$ FW) was recorded in saline-alkali treatment in comparison to control (3 $mg \cdot g^{-1}$ FW). Fruit DW and the contents of dry matter, TSS, and OA were higher in winter crop than summer crop in all stress treatments. These results show that, in spite of very low EC in saline-alkali treatment, soluble sugar and DM contents were equal to that of high EC treatments. This data suggest that rhizospheric pH may also have a role in influencing SC of tomato fruits.

Chapter – 5

5. General Discussion

Tomato has evolved the most prized vegetable not only because of its acidic-sweet taste, but also due to its richness in healthy nutrition and antioxidants. This could be the prime reason that demand for tomato with high solid content is high, round the year, in Japan and elsewhere. Since water is the major component of tomato fruits and fruit solid content accounts only for 5-7 % on fresh weight basis (Davies and Hobson, 1981), growers struggle to produce fruits of high solid content to meet the consumers demand. Moreover, processing industry also prefers fruits with low water content because of its high processing efficiency and low processing cost. Therefore, in this study, the regulation of solid accumulation in tomato fruits, as influenced by the sucrose concentration of the phloem sap due to the manipulation of leaf to fruit ratio or subjecting plants to saline-alkali stress, was examined.

Some of the previous studies have shown that dry matter accumulation in fruits is regulated sink-dependently (Heuvelink and Buiskool, 1995; Marcelis, 1993b; Valantin et al., 1999). However, another work reported that tomato genotype with high leaf area per fruit had high solid content in fruit (Hewitt and Stevens, 1981), suggesting that SC of fruits could be regulated source-dependently. The present study in Chapter-2 explained that dry matter accumulation in tomato fruits may be sink-dependent but the contents of dry matter and soluble solid are regulated source-dependently via the regulation of the sucrose concentration of phloem sap. Manipulating leaf to fruit ratio (LFR) within the range of 0.2 to 3, on a single source-sink unit, indicated that fruit dry weight accumulation was increased up to the LFR of 1 (3.5g - 7g), further increase in LFR did not increase fruit dry weight. Saturation of DW at LFR 1 suggested that accumulation of dry matter in fruit is strictly regulated sink dependently unless the LFR is not below the threshold capacity to

support fruit growth. In contrast to DW, the contents of dry matter, total sugar, and organic acids on a fruit fresh weight basis increased linearly within the whole range of LFRs (0.2-3). Interestingly, from LFRs of 1 to 3, the contents of fruit dry matter, sugar, and organic acids increased by 40-50%, though fruit dry weight was saturated in this range of LFRs.

These observations suggest the association of fruit soluble solid with the sucrose concentration of the phloem sap entering into fruits. Import of water into tomato fruits is regarded via phloem (Ho et al., 1987; Araki et al., 2004; Plaut, et al., 2004), along with sucrose assimilated in leaves (Walker and Ho, 1977). Therefore, sucrose concentration of the phloem sap, which may change according to alteration in LFR, may affect the solid content of fruits; but the relationship between sucrose concentration of phloem sap and fruit solid content has never been investigated. Phloem and xylem exudations were collected in EDTA solution from the cut pedicel of tomato fruits. The result showed that sucrose concentration in the phloem sap was increased from 4% to 7% by increasing LFR, which also increased the contents of dry matter and total sugar in fruits. The contents of fruit dry matter and total sugars showed a positive correlation with the concentration of sucrose in phloem sap. This data suggest that, while dry weight accumulation in tomato fruit is regulated sink dependently, the contents of dry matter and sugar are regulated source dependently via the sucrose concentration of phloem sap.

Besides source-sink manipulation, saline stress treatment having high EC is also reported to increase fruit solid content (Ho et al., 1987; Gao et al., 1998; Sakamoto et al., 1999; Saito et al., 2009). Salinity is a serious soil problem throughout the world, but world arable land has another problem of saline-alkali/sodic soil conditions which affects larger land area than salinity (Tanji, 1996). Such soils are characterised by high pH in addition to high EC. The influence of salinity (high EC) on increasing fruit solid content is reported

recurrently (Ho et al., 1987; Mitchells et al., 1991a; Yin et al., 2010), but the affect of saline-alkali stress (high pH as well as EC) on the content of fruit solid has never been studied so far. Understanding the influence of saline-alkali stress on fruit solid accumulation may help us improve fruit quality as well as broaden the existing knowledge for the genetic improvement of new varieties to exploit such stress conditions.

Growing tomato plants in saline-alkali conditions (0-120 mM $NaHCO_3$) showed that fruit fresh weight, cross sectional area, and dry weight was not affected within the range of 0-90 mM stress, whereas the contents of fruit dry matter, total sugar, and organic acids were increased significantly (Chapter-3). These results indicate that saline-alkali stress, in spite of high pH, can improve solid content in tomato. High solid content in tomato fruits under saline stress is supposed to be either due to enhanced transport of assimilates to fruits from source leaves (Gao et al., 1996; Saito et al., 2009), or reduction in water imported into fruits (Ho et al., 1987; Sakamoto et al., 1999). In the present study, fruit fresh weight and dry weight were not affected significantly but the contents of dry matter, sugar, and organic acids were increased significantly by saline-alkali treatment. Therefore, it can be assumed that assimilates accumulation was stimulated in fruit.

This work indicated that saline-alkali stress can improve the contents of fruit dry matter, sugar, and organic acids. Whether this increase was due to the influence of high EC or pH of the root-zone is not clear from the study of Chapter-3. Application of sodium bicarbonate salt increased soil leachate pH as well as EC as compared to control. Although, fruit total sugar revealed correlation to both EC and pH of the soil leachate, soil leachate EC and pH also revealed correlation. Therefore, it was not clear from this experiment (Chapter-3) whether soil pH plays a role in increasing solid content in fruits or not. To understand the role of soil pH in increasing solid content in fruits, further experiments were conducted.

In new set of experiments, tomato plants were subjected to equal concentration of $NaHCO_3$, NaCl, and mixtures of these salts in comparison to control treatments in different seasons. Fruit cross sectional area, fresh weight, dry weight, the contents of total sugar, and dry matter were affected similarly in saline-alkali and saline treatments instead of two times lower EC in saline-alkali treatment than saline treatment (Chapter-4). Li et al. (2001) reported that solid content in tomato fruits is increased in response to increasing EC of the root-zone, whereas relative fruit growth was negatively correlated to root-zone EC. Moreover, Ehret and Ho, (1986b) and Reina-Sanchez et al. (2005) reported water uptake in tomato plant is in inverse relationship to root-zone EC. Regarding different EC among treatments, if only rhizospheric EC influenced fruit SC, fruits in saline-alkali treatments which had lower soil leachate EC (4-6 $dS \cdot m^{-1}$)must have lower solid content or must have higher fruit fresh weight than the salt treatments with high soil leachate EC (10-12 $dS \cdot m^{-1}$). Nonetheless, no statistically significant difference was observed among fruits from saline and saline-alkali or the mix-salt treatments in fruit fresh and dry weight as well as solid content and total soluble sugar. Therefore, the results suggest that fruits solid content may be increased not only by high EC of the root-zone, but also by high rhizospheric pH. Moreover, increase in the organic acids content in saline-alkali treatment than other treatments indicate it could be a special feature of saline-alkali stress, as is observed in the roots and shoots of some other crops (Yang et al., 2007; Guo et al., 2010). These results suggest that in saline-alkali treatment, apart from EC, high soil pH may have decreased water accumulation in fruits and thereby increased the contents of dry matter, sugar, and organic acids.

In conclusion, the present study explains the relationship between the LFR and the fruit solid content, as well as the impact of saline-alkali stress and the role of high rhizospheric pH in increasing the contents of fruit dry matter and soluble solids, in tomato.

In tomato fruits, the contents of dry matter and soluble solids can be increased by an increase in LFR. Such increase in fruit solid content appears due to the increase in the concentration of sucrose in the phloem sap entering into fruits. Moreover, saline-alkali stress can increase the accumulation of solid, sugar, and organic acids in fruits as is observed in saline stress. Saline-alkali stress improved fruit solid content in spite of low EC than saline stress, which suggest that high soil pH play a role in increasing the contents of fruit solid and soluble solids.

Chapter – 6

6. Thesis summary

Tomato (*Solanum lycopersicum* L.) is a highly nutritious vegetable and remains in high demand round the year throughout the world. Like other fleshy fruits, water is the major component of tomato, while the solid portion is chiefly composed of sugar, organic acids, amino acids, vitamins, and minerals. Consumers prefer fruit with high contents of sugars, organic acids, and amino acids owing to good taste and health-benefits concerns. Moreover, processing industry also prefers fruits with low water content because of its high processing efficiency and low processing cost. Solid contents of fruit, therefore, not only define its nutritional value but indicate consumer preference and processing efficiency as well.

Solid content of fruits may be manipulated through modifying growing conditions, changing leaf to fruit ratio (LFR), or introgression of desirable traits from the wild relatives into the cultivated species. It is often reported that the accumulation of dry matter in fruits is regulated sink-dependently. Whereas some work suggests that the content of soluble solids in fruits is increased with high LFR or CO_2 enrichment, and therefore is source-dependently. In tomato most of the water transported into fruits is suggested via the phloem pathway, along with sucrose. Therefore, possibly changing LFR on a plant may change the concentration of sucrose in phloem sap and thereby soluble solid in fruit. On tomato plants, except the first truss, each fruit truss is preceded by usually 3 leaves which supply assimilates for the fruit growth on that truss. Fruit growers usually keep 4-5 fruits on a single truss, but to improve the solid content of fruit, source-sink balance must be properly maintained. Therefore, the relationship among LFR, sucrose concentration of the phloem sap, and fruit solid content is of high importance.

Soil salinity is a serious problem in many tomato growing countries. Exposure of plants to saline stress has long been shown to increase fruit solid content. However, problem of saline-alkali soil or bicarbonate-rich irrigation water is more widespread than salinity, especially in regions like Asia, Pacific, and Australia. These soils have high pH along with high EC. The exclusive influence of high rhizospheric EC on the content of fruit solid have been studied extensively, but the combined effect of high pH and EC on fruit solid content has never been studied before. If saline-alkali stress also improves the accumulation of solid in tomato fruits; whether soil pH, besides EC, also play a role in this improvement will be very interesting to know.

In this study, we firstly focused on the relationship between the sucrose concentration of the phloem sap and fruit final solid contents under different leaf- fruit ratio. Secondly, we attempted to understand the impact of saline-alkali stress on the accumulation of solid in fruits and the possible role of soil pH in the response.

Relationship between fruit solid content and the sucrose concentration of the phloem sap at different source-sink ratios in tomato

Regarding the previous observations that more than 90% of the water is translocated via the phloem into a fruit in tomato, soluble solid content of fruits may be strongly influenced by the sucrose concentration of the phloem sap entering into fruits. In this experiment, the relationship of fruit soluble solid contents and the sucrose concentration of the phloem sap in tomato at various source-sink ratios (0.2, 0.4, 0.6, 0.75, 1, 2 and 3 LFR) was evaluated. Fruit fresh weight (FW) was 40 g at the lowest LFR and increased to 80 g at LFR of 1, but no obvious change was observed at higher LFRs. Fruit dry weight (DW) increased linearly ($r=0.930$) from 3.5 g to 7 g when LFR was increased from 0.2 to 1, indicating source dependent regulation of dry matter accumulation. However, it did not change when the LFR was increased beyond 1, indicating that dry matter accumulation was not affected by

the source at high LFRs.

By contrast, the contents of fruit dry matter (DM), total soluble sugars (TSS), and organic acids increased linearly within the whole range of LFR from 0.2 to 3 (r= 0.972, 0.890, and 0.943, respectively). Using these plants, phloem sap was collected from the cut end of the pedicle in EDTA solution. The sucrose concentration of the collected phloem sap showed a positive correlation to fruit dry matter contents (r=0.930). Contents of fruit total sugars also correlated positively with the phloem sucrose concentration (r=0.900). This data suggested that, while dry matter accumulation per fruit is not affected by the source above LFR of 1, the content of soluble sugars and dry matter on a fresh weight basis is determined source dependently via the sucrose concentration of the phloem sap.

Impact of saline-alkaline stress on the accumulation of solids in tomato fruit

Growing of tomato plants in saline conditions is often reported with high solid content in fruits. Saline-alkali soil, unlike saline soil, has high pH besides high EC. Growing of tomato plants in such conditions may influence fruit solid content or not is not reported. Therefore, this experiment was performed to investigate the role of saline-alkali stress (0-120 mM) in SC accumulation in tomato fruits. Addition of sodium bicarbonate ($NaHCO_3$) to plants increased pH of soil leachate in 90 and 120 mM stress treatments (above pH 8) in comparison to 0 mM treatment (about pH 6), just two weeks after the salt application. Similarly, soil leachate EC increased to 5 and 6 $dS \cdot m^{-1}$ at 90 and 120 mM respectively, but in control (0 mM) plants EC gradually decreased from 4 to 1.5 $dS \cdot m^{-1}$.

Saline-alkali stress did not decrease fruit size and FW within the range of 0-90 mM, except at 120 mM treatment. Likewise, $NaHCO_3$ application had no effect on fruit DW. However, DM content increased significantly from 6.8% at 0 mM treatment to 8.5% at 90 and 120 mM treatments. Total soluble sugar content increased to 3% in 90 mM treatment in comparison to 2% in the control, but starch content remained the same. The increase in

total soluble sugar was due to significant accumulation of hexose as well as sucrose in ripe fruits. In addition to carbohydrates, saline-alkali stress influenced organic acids accumulation as well. Citric acid, being the major acid, was significantly higher than control at stress level of higher than 30 mM. These results show that saline-alkali stress (0-90 mM) can increase the solid content of fruit without reducing fruit weight.

Comparative study on the accumulation of solids in tomato fruit under saline-alkaline and saline stress conditions during different growing seasons

Increasing number of evidence suggest the role of high rhizospheric EC in enhancing SC of tomato fruit. Data in previous chapter showed that saline-alkali stress, retaining high pH in addition to high EC in the medium, can improve SC of tomato fruits. To elucidate the role of soil pH in improving the SC of fruits, tomato plants were grown in saline-alkali ($NaHCO_3$), saline (NaCl), and mix-salt ($NaHCO_3$+NaCl) conditions in two different seasons.

Results indicated that saline-alkali treatment increased soil-leachate pH (8.5), while pH was similar in saline and control treatments (pH 6). On the contrary, soil-leachate EC was two times lower (5-6 $dS \cdot m^{-1}$) in saline-alkali treatment than saline treatment (11-13 $dS \cdot m^{-1}$). Fruit fresh weight and dry weight decreased in all treatments to similar extent. Dry matter content of fruits increased significantly from 7 to 14% and TSS from 3 to 5% in all treatments. The content of sucrose on a fresh weight as well as dry weight basis increased in all stress treatments. Organic acids accumulated in stressed fruit higher than control, but their content was higher particularly in saline-alkali treatment. Stress treatments increased citric acid content but the maximum content (8 $mg \cdot g^{-1}$ FW) was recorded in saline-alkali treatment in comparison to control (3 $mg \cdot g^{-1}$ FW). Fruit DW and the contents of dry matter, TSS, and OA were higher in winter crop than summer crop in all stress treatments. These results show that, in spite of very low EC in saline-alkali

treatment, soluble sugar and DM contents were equal to that of high EC treatments. This data suggest that rhizospheric pH may also have a role in influencing SC of tomato fruits.

In conclusion, the present study explains the relationship between the LFR and the fruit solid content, as well as the impact of saline-alkali stress and the role of high rhizospheric pH in increasing the contents of fruit dry matter and soluble solids, in tomato. In tomato fruits, the contents of dry matter and soluble solids can be increased by an increase in LFR. Such increase in fruit solid content appears due to the increase in the concentration of sucrose in the phloem sap entering into fruits. Moreover, saline-alkali stress can increase the accumulation of solid, sugar, and organic acids in fruits as is observed in saline stress. Saline-alkali stress improved fruit solid content in spite of low EC than saline stress, which suggest that high soil pH play a role in increasing the contents of fruit solid and soluble solids.

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