Increase in Total Soluble Solids Content in Tomato Fruits by Applying Water Stress in High Density-Low Truss Cultivation

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ABSTRACT

This study was conducted to increase sugar content in tomato fruits by applying water stress in high density-low truss cultivation. In the first chapter, the change in total soluble solids content (TSS) in fruits as affected by high electrical conductivity (EC) levels up to 13.7 dS m^{-1} of nutrient solution at different growth stages and incidence of blossom-end rot (BER) as affected by salt stress intensity and growing seasons were investigated. Growth of tomato plants was inhibited and fruit yield decreased, while

soluble sugar content in fruits increased as EC of nutrient solution increased. The sugar content of tomato fruits cultivated in summer season was higher than that cultivated in winter season. BER incidence was less in winter cultivation. In the second chapter, the change in fruit quality as affected by combination of salt stress and root zone restriction was investigated to reduce the negative side effects of high EC treatment such as growth inhibition, yield loss, and higher incidence of BER. Growth and yield decreased as EC level increased and root zone volume decreased. TSS, titratable acid, and ascorbic acid in fruits increased as the root zone volume decreased. TSS increased in higher EC treatment ($\geq$ 4.2 dS m^{-1}) and TA remarkably increased in the EC treatment of 7.0 dS m^{-1} those combined with the severe root zone restriction (container size S treatments). Glucose, fructose, citric acid, and ascorbic acid contents in tomato fruits as affected by EC level and root zone restriction were most significant when the treatments was applied at the fruit-ripening stage. Results imply that TSS in tomato fruits increased as EC levels increased and the effect is more significant when the high EC treatment is applied at the earlier stages. Fruit quality could be improved with minimized yield reduction and incidence of BER if the high EC treatment was combined with root zone restriction treatment. Cultivating tomato plants using a 200 mL-container with application of high-EC nutrient solution will be feasible for high density-low truss cultivation of high quality tomato fruits.

Keywords: high density-low truss cultivation, root zone restriction, salt stress, total soluble solids content, water stress

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INTRODUCTION

Since tomato (*Solanum lycopersicum* L.) is one of the most important horticultural crops in the world, and consumers demand varieties of higher quality, strategies focused on increasing fruit quality continue to be of great interest (Dorais et al., 2001; Gruda, 2005, 2009). Water is an important factor influencing yield and fruit quality. A certain degree of water stress; such as deficit irrigation (DI), salt stress, and root zone restriction improves the fruit quality but it reduces plant growth, fruit yield, and caused blossom-end rot (BER) (Favati et al., 2009; Kirda et al., 2004; Mitchell et al., 1991; Patanè and Cosentino, 2010; Zegbe-Domínguez et al., 2003) at the same time. Yield reduction and the greater incidence of BER of tomato fruits may cause, moreover, economic losses in some seasons under water stress conditions. DI reduces fruit water accumulation and fresh fruit yield, but irrigation with saline water has less effect than DI on total fresh fruit yield (Mitchell et al., 1991). Total soluble solids content in ripe fruits increases with salinity and hence the use of moderately saline irrigation water is recommended to improve fruit quality (Mizrahi et al., 1988), however, special care must be taken when using saline water in a commercial crop as from electrical conductivity (EC) above 2.5 dS m^{-1} a more than 10% yield reduction per additional dS m^{-1} unit is expected (Saranga et al., 1991). Tomato fruits from the root zone restriction systems were superior in a few

quality parameters including total soluble solid, but generally the differences were small (Thybo et al., 2005, 2006).

The high density-low truss (HDLT) cultivation is one of the simplest growing systems, which may especially have advantages fora year-round production in large scale with labor saving mainly because of its short period of cultivation comparing to the multiple-truss cultivation (Cooper et al., 1966; Giacomelli et al., 1994; Hisatomi and Fujimoto, 1978; Kobayashi et al., 1997; Sasaki et al., 1991). In HDLT cultivation system of tomato growing, the main shoot is pinched, leaving a few leaves above 1-3 trusses, and only the fruits in the 1st to -3rd trusses are harvested. The HDLT cultivation has a great advantage with respect to management of the composition of the nutrient solution without extensive consideration of its long-term effects on plant growth; this former technique aids the production of high quality tomatoes. In this cultivation, some treatments for the enrichment of fruit quality such as salt stress and root zone restriction treatments are easily extended to the plants irrespective of the long term effects on the growth.

The objectives of this study were to investigate the change in TSS in tomato fruits, yield, and incidence of BER as affected by high EC levels of nutrient solution at different growth stages and growing seasons (Chapter 1), and to investigate the changes in fruit quality as affected by combination of salt stress and root zone restriction (Chapter 2) in the HDLT tomato

cultivation.

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CHAPTER 1

Total Soluble Solids Content in Tomato Fruits as Affected by High EC Levels at Different Growth Stages

INTRODUTION

Total soluble solids content (TSS, commonly measured using a refractometer) is the most common flavor index associated directly with sugar and organic acid concentrations in the tomato fruits (Stevens et al., 1977; Young et al., 1993). Much research has been conducted for enhancing TSS under high electrical conductivity (EC) (Adams, 1991; Cornish, 1992; Krauss et al., 2006; Lin and Glass, 1999; Mitchell et al., 1991; Wu et al., 2004). Effect of EC on TSS and its mechanism seem to be generally understood as an osmotic effect and a resulting reduced water flux to the fruits.

The summer production of fresh tomatoes grown in glasshouses usually shows a yield reduction, together with an increase in blossom-end rot (BER) and cracking, but an increase in dry matter and sugar content promoting fruit quality (Winsor and Adams, 1976). BER and cracking are

physiological disorders of tomato, both relating to water import into the fruits. Fresh tomatoes are produced year-round in the greenhouse under contrasting environmental conditions, triggering seasonal variations in their flavor and nutritional quality, e.g., fruit quality fluctuates with the TSS, which generally increases during summer and decreases during winter. (Anza et al., 2006; Davies et al., 1981; Grierson et al., 1986; Raffo et al., 2006; Toor et al., 2006).

The objectives of this study were to investigate the change of TSS in tomato fruits as affected by high EC levels of nutrient solution at different growth stages and physiological disorders as affected by salt stress intensity and growing seasons.

1

MATERIALS AND METHODS

Plant materials and culture conditions

'Rafito' tomato seeds (De Ruiter Seeds C.V., Bleiswijk, The Netherlands) were sown on 50-cell tray filled with growing media (Sikmulsekye; Nong Woo Green-Tech Co., Ltd., Yeoju, Korea) on June 28, 2010 (summer season) and September 11, 2010 (winter season), respectively, and subsequently cultured in growth chambers equipped with white fluorescent lamps. After cotyledons were fully unfolded (7 days after sowing (DAS)), the plants were sub-irrigated once a day with the half-strength modified Yamazaki nutrient solution for tomato (EC 0.7 dS m^{-1} and pH 6.0). At the first stage (14 to 30 DAS), the nutrient solutions with EC levels of 0.7, 3.7, 6.7, and 13.7 dS m^{-1} were applied (Fig. 1-1). At 30 days after sowing, tomato seedlings were transplanted to Wagner pots filled with perlite and grown hydroponically in the greenhouse at the Experimental Farm (37°16′N, 126°59′E) of Seoul National University, Suwon, Korea. The nutrient solution was supplied using a drip irrigation system and 2 days after transplanting, the EC levels were maintained or increased to 2.7 (control), 3.7 (L), 6.7 (M), and 13.7 (H) dS m^{-1} at the second (summer season; 32 to 80 DAS, winter season; 32 to 88 DAS) and the third (summer season; 80 to 126 DAS, winter season; 88 to 167 DAS) growth stages. During cultivation,

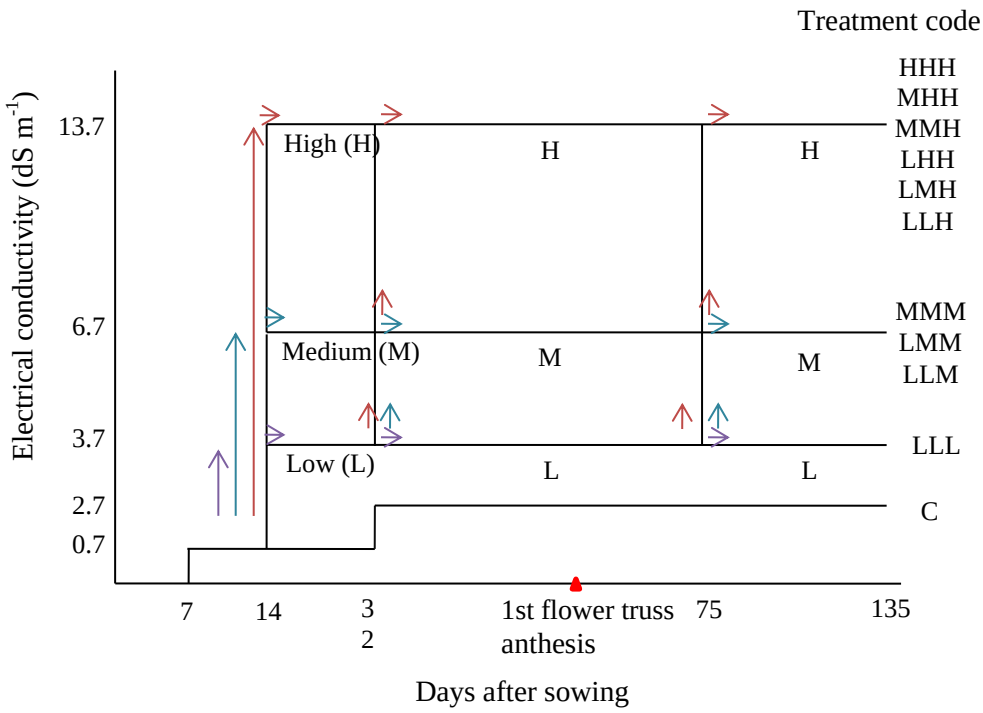

Fig. 1-1. Treatment codes with different EC levels at various growth and development stages.

all side shoots and lower foliage were removed and growing points were pinched off allowing three leaves above the second cluster.

Nutrient solution preparation

EC was adjusted at 2.7, 3.7, 6.7, and 13.7 dS m^{-1} by diluting and mixing the concentrated nutrient stock solutions A and B with tap water, where solution A contained 101 g L^{-1} KNO_3, 177 g L^{-1} $Ca(NO_3)_2 \cdot 4H_2O$, and 7.65 g L^{-1} Fe-EDTA, and solution B contained 101 g L^{-1} KNO_3, 38 g L^{-1} $NH_4H_2PO_4$, 123 g L^{-1} $MgSO_4 \cdot 7H_2O$, 570 mg L^{-1} H_3BO_3, 405 mg L^{-1} $MnSO_4 \cdot 4H_2O$, 45 mg L^{-1} $ZnSO_4 \cdot 7H_2O$, 20 mg L^{-1} $CuSO_4 \cdot 5H_2O$, and 5 mg L^{-1} $Na_2MoO_4 \cdot 2H_2O$.

Analysis of total soluble solids content

TSS in tomato fruits was determined by a digital refractometer (Atago PAL-1, Atago Co., Ltd., Tokyo, Japan).

Statistical analysis

Data were analyzed using SAS 9.1 version (SAS Inst. Inc., Cary, NC, USA) for LSD test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Plant growth

Plant height was lowest in the HHH treatment in both growing seasons (Tables 1-1, 1-2). Stem diameter in the treatments LHH, MHH, and HHH measured at 80 and 126 DAS in summer and at 88 and 167 DAS in winter seasons were significantly lower than that in other treatments. The plant height and stem diameter were mainly affected by high EC level at the first or second stages. Dumbroff (1974) reported that early exposure to salt stress restricted the tomato plants more than prolonged exposure beginning later in the developmental process.

In the present study, tomato plants showed growth reduction when exposed salinity. Plant growth such as plant height and stem diameter reduction is generally observed in plants exposed to salinity stress (Bolarin et al., 1991; Cruz et al., 1990; Mahajan and Tuteja, 2005; Munns, 2002; Munns et al., 2008). This may be partly due to lower water potential in the cells which, in turn, causes stomatal closure and limits CO_2 assimilation (Amirjani, 2011).

Days to flowering of first flower truss and second flower truss were not only delayed as EC levels increased in both growing seasons but also more delayed in all the treatments in winter season (Tables 1-1, 1-2). These results agree with Blits and Gallagher (1991) and Stanton et al. (2000) finding

Table 1-1. Plant height, stem diameter, and days to flowering in tomatoes as affected by different EC levels at various growth stages in summer season. Treatment codes are indicated in Fig. 1-1.

Treatment	Plant height[z] (cm)	Stem diameter (mm)		Days to flowering	
		80 DAS	126 DAS	First flower truss	Second flower truss
Control	156.2 a	9.8 a	14.5 a	53.2 b	61.0 e
LLL	143.2 bc	9.8 a	14.4 ab	51.3 b	60.5 e
LLM	150.2 ab	9.0 ab	13.1 bcd	50.5 b	61.3 e
LLH	140.0 cd	9.3 ab	13.1 bcd	51.4 b	62.4 de
LMM	138.9 cd	9.0 ab	13.2 abc	53.0 b	65.7 cd
MMM	138.9 cd	9.0 ab	13.4 abc	51.8 b	64.7 cde
LMH	131.7 d	8.4 b	11.8 d	50.2 b	60.8 e
MMH	134.5 cd	9.2 ab	12.8 cd	53.3 b	64.2 cde
LHH	110.5 e	8.6 b	9.1 e	58.5 a	68.3 bc
MHH	112.3 e	7.3 c	9.6 e	59.0 a	71.8 ab
HHH	97.9 f	7.3 c	7.6 f	60.4 a	74.2 a

[z]Mean separation within columns by LSD test at $P = 0.05$.

Table 1-2. Plant height, stem diameter, and days to flowering in tomatoes as affected by different EC levels at various growth stages in winter season. Treatment codes are indicated in Fig. 1-1.

Treatment	Plant height[z] (cm)	Stem diameter (mm)		Days to flowering	
		88 DAS	167 DAS	First flower truss	Second flower truss
Control	147.5 a	11.3 ab	15.7 a	47.7 e	62.7 de
LLL	130.4 cde	10.8 abc	12.7 bc	45.7 e	58.8 ef
LLM	131.8 cde	11.5 a	13.5 b	51.8 de	55.2 f
LLH	136.8 bcd	10.1 abcd	12.1 bcd	64.2 bc	66.3 cd
LMM	139.4 abc	9.9 bcd	12.8 bc	57.8 cd	50.3 e
MMM	142.5 ab	8.8 edf	12.7 bc	59.2 ab	80.8 a
LMH	137.7 abc	9.5 cde	11.2 cd	54.5 d	68.2 c
MMH	127.0 de	10.3 abc	11.3 cd	71.8 a	80.8 a
LHH	123.8 e	8.1 f	10.4 d	64.3 bc	75.3 b
MHH	122.2 e	8.3 ef	11.7 bcd	72.0 a	81.7 a
HHH	110.4 f	6.5 g	8.2 e	70.5 ab	79.8 ab

[z]Mean separation within columns by LSD test at $P = 0.05$

a delay in flowering with increased salinity levels. Salinity exerts multiple stresses on plants, including osmotic imbalance, nutritional deficits, and cellular toxicity (Levitt, 1980; Wang et al., 2001), it may be difficult to identify the specific mechanisms that alter life history shifts in important traits such as flowering phenology (Peter et al., 2002).

Fruit diameter, yield, and incidence of BER

Fruit diameter of tomatoes was highest in control and the treatment LLL, and smallest in the treatment LHHin summer season and in the treatment HHH in winter season (Fig. 1-2). Fruit diameter of tomatoes showed similar trend to both growing stages, but fruit diameter of tomatoes was smaller in summer season than in winter season each treatment. Results agree with Estan et al. (2005) reported a decrease in the fruit size has been observed in salt-treated tomato plants. Yield in winter season was higher than in summer season overall (Fig. 1-3). The larger reduction in fruit yield in summer season resulted from the higher incidence of BER of fruits (Fig. 1-4). The summer production of fresh tomatoes grown in glasshouses usually shows a yield reduction, together with an increase in BER (Winsor and Adams, 1976). Yield showed similar trend to fruit diameter of tomatoes because yield was affected by fruit size. The highest yield of tomato was obtained in control in both growing seasons, but the yield of tomato in the treatment LLL was similar with control treatment in winter season.

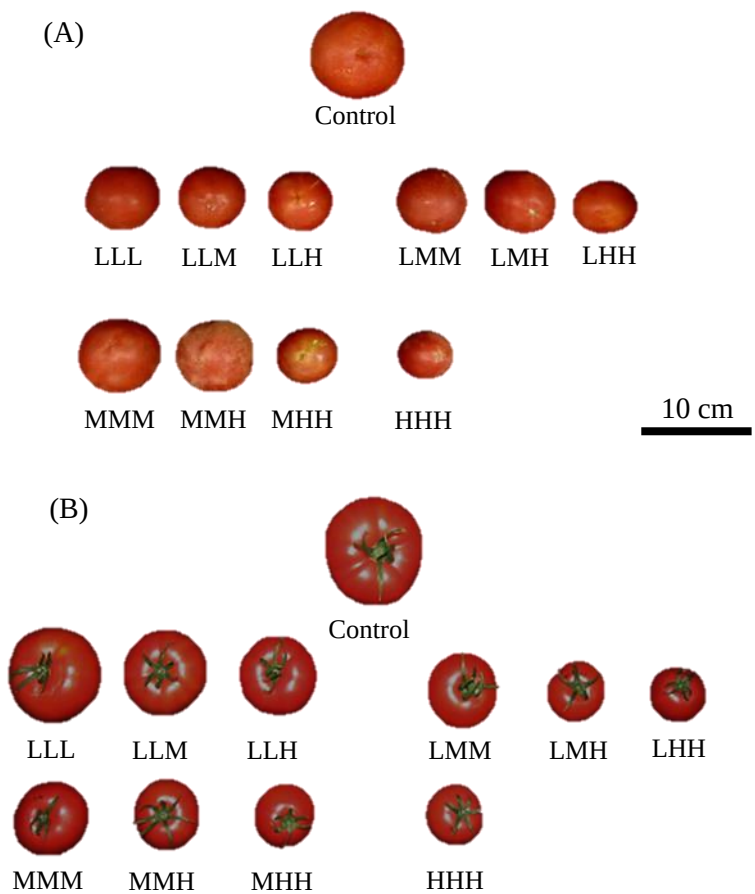

Fig. 1-2. Tomato fruits as affected by different EC treatments in summer (A) and winter (B) seasons. Treatment codes are indicated in Fig. 1-1.

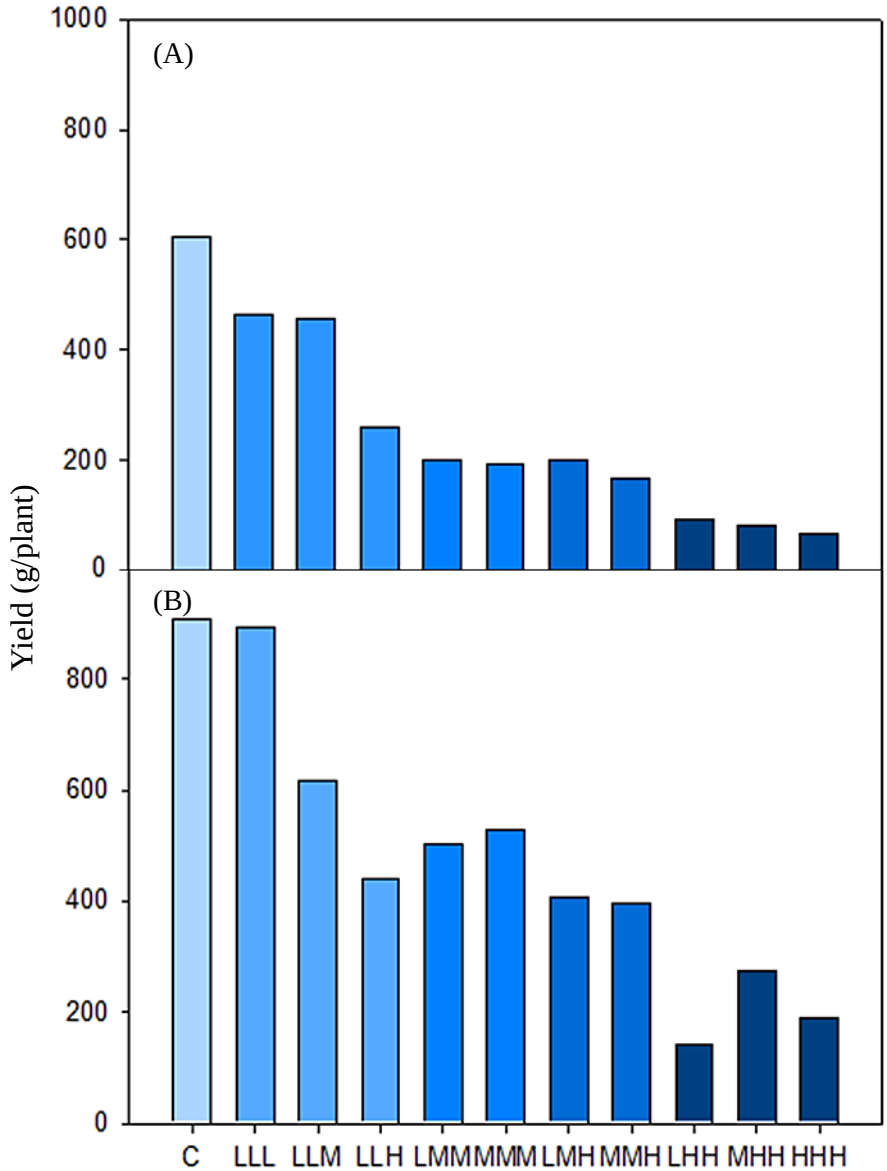

Fig. 1-3. Fruit yield of tomato as affected by different EC levels at various growth stages in summer (A) and winter (B) seasons. Treatment codes are indicated in Fig. 1-1.

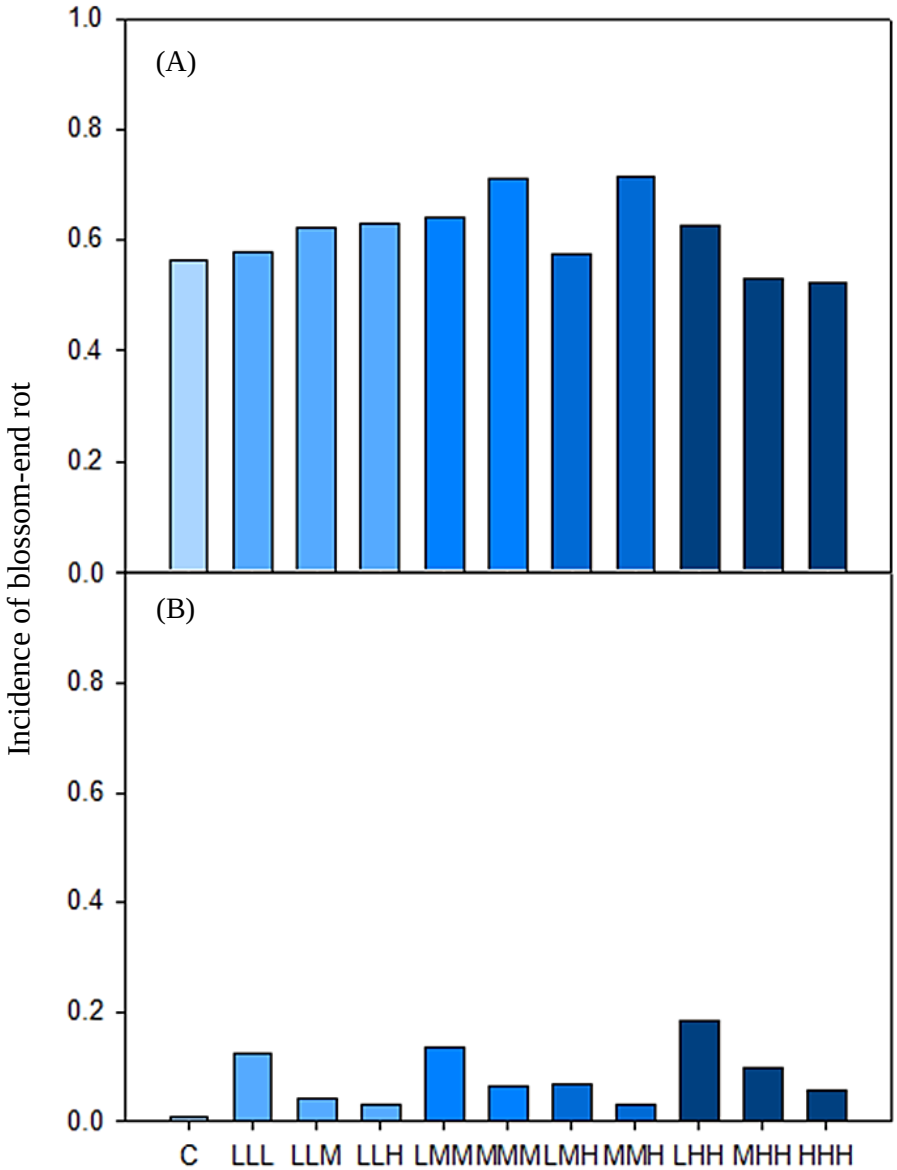

Fig. 1-4. Incidence of blossom-end rot as affected by different EC levels at various growth stages in summer (A), and winter (B) seasons. Treatment codes are indicated in Fig. 1-1.

Magan et al. (2008) reported that evaporative demand appears to have been consistently above the level below which tomato fruit yield is unaffected by EC 2.6 to 4.0 dS m^{-1}, as has been observed during cool season. Yield showed a tendency to gradually decrease as EC level increased at second or third stages (EC levels same with the first and the third stages, or the first and the second stages) in both growing seasons, especially in winter season. Yield of tomato was pretty similar as EC level changed at the first stage (EC levels same with the second and the third stages) in both growing seasons. In the treatments LHH, MHH, and HHH (those were affected by the highest EC level after transplanting) yield were lower than other treatments in both seasons. These results show that yield reduction was mainly affected by high EC levels of nutrient solution supplied after transplanting (flowering and fruit set) and agree with Helyes et al. (1994) finding yield of tomatoes are sensitive to water stress after transplanting especially flowering and fruit set.

Incidence of BER was increased as the EC levels increased in winter season; especially the highest was in the treatment LHH where EC level was elevated from low level (even though its EC level was higher than in control treatment) to highest level after transplanting. Incidence of BER in summer was higher than in winter season overall.

Calcium is transported through the transpiration stream (Tibbitts, 1979) and it is implicated in the BER incidence in tomato fruits (Barker and Ready,

1994). Adams and Ho (1992) summarized that water stress such as salt stress, which reduces Ca^{2+} uptake, was the most common cause of BER. Ehret (1986) and Belda (1993) reported that restricted transport of Ca^{2+}, e.g. due to substantially retarded xylem tissue development in the pedicel and within the fruits at high salinity. High temperature may act both by reducing stress tolerance because of a promotion of gibberellin activity together with high irradiance, and by increasing stress (Nilsen and Orcutt, 1966), especially when occurring during rapid fruit development (Ho et al., 1993; Sonneveld and Voogt, 1991). An increased incidence of BER associated with high temperature and rapid growth rate in plants grown at high salinity in summer has been confirmed by Wui and Takano (1995). Indeed, competition for Ca^{2+} between leaves and fruits is known as distraction Ca^{2+} flux from the fruits to the leaves at high transpiration in summer (Gerard and Hipp, 1968; Ho, 1989).

TSS content

TSS in tomato fruits in winter season was higher than that in summer season overall, and the highest was 11.7 ^{o}Brix in the treatment HHH (Fig. 1-5). Contrary to a tendency to yield of tomato decreases, TSS increased as EC level increased at second or third stages (EC levels same with the first and the third stages, or the first and the second stages) in both growing seasons. Increase in TSS as the result of sugar condensation due to reduced

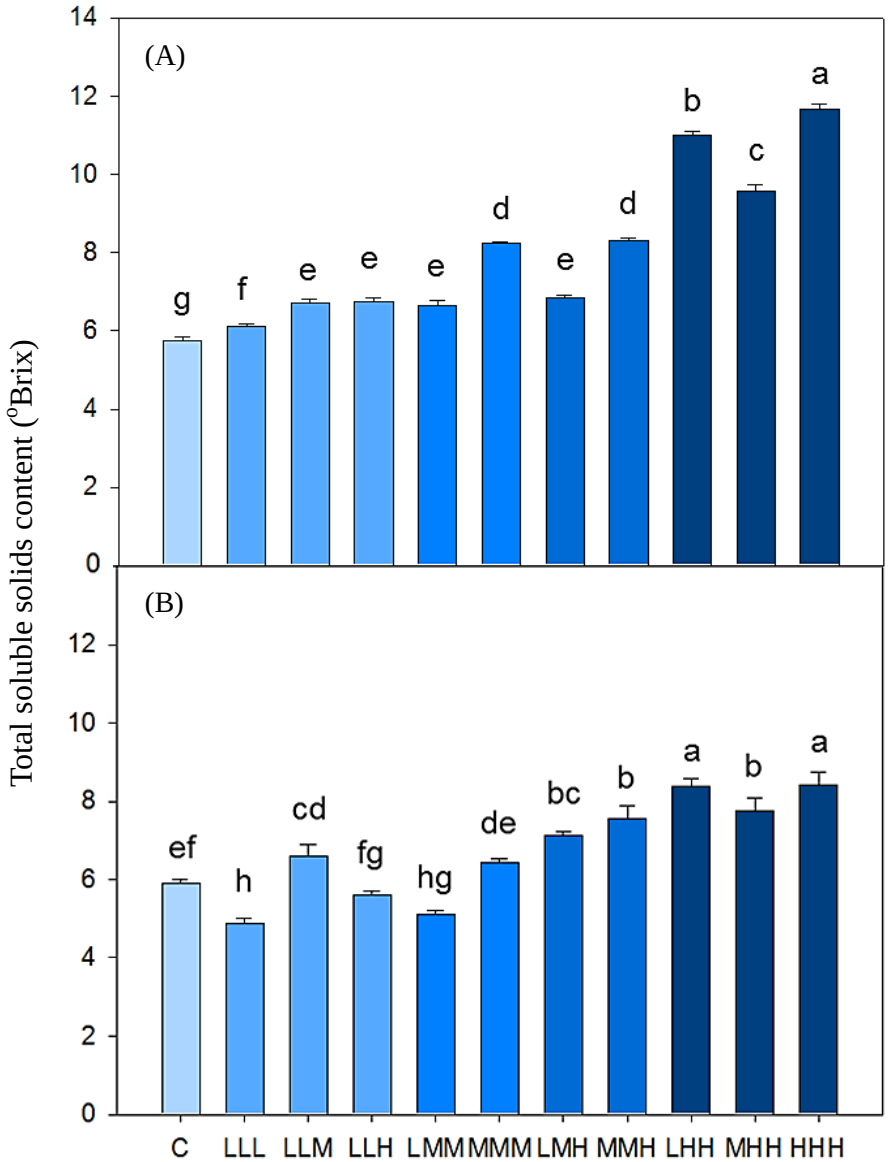

Fig. 1-5. Total soluble solids content in fruits of tomato plants as affected by different EC levels at various growth stages in summer (A) and winter (B) seasons. Treatment codes are indicated in Fig. 1-1.

water uptake with decreased fruit size at salinity treatment in the present study have been consistently observed in other studies (Adams, 1995; Cornish, 1992; Cuartero and Fernandez-Munoz, 1999; Eltez et al., 2002; Krauss et al., 2006; Lin and Glass, 1999; Mitchell et al., 1991; Sonneveld and van der Burg, 1991; Wu et al., 2004).

Results indicate that TSS increasing was mainly affected by high EC level after transplanting, and increasing the EC level can increase TSS up to 11.7 ^{o}Brix while yield decrease and increasing incidence of BER. According to Dorai et al. (2001) and Maggio et al. (2004), the negative effects of salinity on fruit yield and BER may be, at least partly, compensated by positive effects on qualitative properties. On negative aspects, studies on how to reduce negative effects increase following water stress maintaining high TSS would be investigated in order to enhance competitiveness.

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CHAPTER 2

Quality Improvement of Tomato Fruits at Different Ripening Stages as Affected by Salt Stress and Root Zone Restriction

INTRODUCTION

Root restriction has been shown to limit plant growth through reductions of growth parameters such as leaf area, leaf number, plant height, and biomass production in various species (Carmi and Heuer, 1981; Liu and Latimer, 1995; Richards and Rowe, 1977) and specifically in tomato plants (Cooper, 1972; Peterson and Krizek, 1992; Ruff et al., 1987). According to the nutrient control theory (Wilson, 1988), a possible cause for the reduction in shoot growth by root restriction is a shortage in supply of nutrients (Bar-Tal et al., 1990; Hanson et al., 1987; Mutsaers, 1983) or water stress (Hameed et al., 1987). However, the effects of root restriction, in most cases, are not implemented through nutrient deficiency (Carmi and Heuer, 1981; Robbins and Pharr, 1988) or water stress (Krizek et al., 1985; Ruff et al., 1987). Nutrient solution culture is a preferred system for studying the direct physiological effects of root restriction, only a few such studies have been carried out (Carmi and Heuer, 1981; Hameed et al., 1987; Peterson and

Krizek, 1992; Robbins and Pharr, 1988), most of which were short-term experiments. Some of these studies analyzed nutrient contents in leaves, but none of them focused on the effects of root restriction on nutrient uptake rate and distribution of nutrients in the entire plant organs. Bar-Tal et al. (1995, 1996) reported that root restriction decreased tomato fruit yield, but reduced the incidence of blossom-end rot (BER) in fruits. Tomato fruits as affected by root zone restriction were superior in a few quality parameters including total soluble solid (TSS; Thybo et al., 2006).

Glucose and fructose account for about 95% of the total sugars in tomato fruits (Davies and Kempton, 1975; Haila et al., 1992; Young et al., 1993), whereas sucrose is detected in trace amounts(Davies and Kempton, 1975; Haila et al., 1992). Among limited information, Petersen et al. (1998) found that the fructose and glucose concentrations in harvested tomato fruits were linearly correlated to the EC of nutrient solution at a range of 3 to 10 dS m^{-1}. Claussen et al. (2006) also reported the increase in glucose and fructose concentration in tomato fruits under nutrient concentration increased by 5.5 times. The sugar composition of tomato fruits is critical because fructose is almost twice as sweet as glucose. Levin et al. (2000) claimed that a transforming tomato with a gene that increases the fructose to glucose ratio can be an approach for flavor improvement in tomatoes.

Lycopene is a powerful antioxidant, which can prevent the initiation or propagation of oxidizing chain reaction (Di Mascio et al., 1989; Nguyen and

Schwartz, 1999; Riso et al., 1999). Lycopene has been reported to have important roles to prevent disease and promote health in humans, usually associated with reducing the risk of cancer and cardiovascular disease (Gerster, 1997; Giovannucci, 1999; Stahl and Sies, 1996). Enhancing lycopene concentration in tomato fruits by manipulating plant growth environments has been reported. For example, lycopene concentration in tomato fruits was reportedly increased by manipulating level of salinity in the nutrient solution (De Pascale et al., 2001; Krauss et al., 2006). Most studies compare lycopene concentration of fruits at red ripeness stage (the fully ripened stage). Since lycopene concentration in tomato fruits increases rapidly during fruit ripening, the concentration should be compared throughout fruit ripening (from late green stage to red stage) rather than the last stage of ripeness to better understand if lycopene synthesis is promoted by water stress. Furthermore, ripening of tomato fruits is accompanied by lycopene synthesis, as chloroplasts are converted into chromoplasts (Fraser et al., 1994; Rhodes, 1980). Little is known about the simultaneous degradation of lycopene during fruit development and ripening, as affected by environmental conditions.

Objective of this study was to investigate the changes in TSS content, titratable acidity (TA), fructose, glucose, organic acids, ascorbic acid (AA), and lycopene in tomato fruits at different ripening stages as affected by combination of salt stress and root zone restriction.

MATERIALS AND METHODS

Plant materials and culture conditions

'Momotaro' tomato seeds (Takii Co., Ltd., Kyoto, Japan) were sown on January 28, 2012 and kept at the air temperature of 28℃ for germination for 4 days in 128-cell plug seedling trays containing a mixture of peat moss and vermiculite (Napura Yodo, Yanmar Agricultural Equipment Co., Ltd., Tokyo, Japan).

Four days after sowing, the trays with germinated seeds were moved to a closed transplant production chamber and sub-irrigation was applied once a day automatically with a commercial nutrient solution (Enshi Standard, Otsuka Chemical Co., Ltd., Tokushima, Japan).

30 days after sowing, tomato seedlings were transplanted to either 189- (S), 608- (M), or 985-mL (L) plastic containers filled with perlite and grown hydroponically in a greenhouse at the Health and Field Sciences of Chiba University, Japan. The plastic containers were made of 4.9, 8.8, and 11.2 cm diameter PVC pipe, respectively, and 1 L d^{-1} of nutrient solution that had EC levels of either 2.4, 4.2, 7.0, or 9.8 dS m^{-1} were drip-irrigated six times per day (Table 2-1). During cultivation, all side shoots and lower foliage were removed and the growing point was pinched off allowing three leaves above the first cluster.

Table 2-1. Description of experimental treatments.

Treatment code	Container volume (mL)	EC (dS m^{-1})
2.4S	189	2.4
4.2S		4.2
7.0S		7.0
9.8S		9.8
2.4M	608	2.4
4.2M		4.2
7.0M		7.0
9.8M		9.8
2.4L	985	2.4
4.2L		4.2
7.0L		7.0
9.8L		9.8

Analysis of TSS content

TSS in tomato fruits was determined using a digital refractometer (Atago PAL-1,Atago Co., Ltd., Tokyo, Japan).

Analysis of TA

TA was determined by titration of the tomato serum with 0.1 N NaOH and was expressed as percent citric acid in the serum.

Extraction and analysis of sugars and organic acids

Fruit sample (2 g) was homogenized with 20 mL of distilled water, filtered using an 8 μm cellulose of the filter paper (Whatman Intl. Ltd., Kent, UK) and filtered again through a 0.45 μm polyvinylidene fluoride membrane of the syringe filter (Agilent Co., New York, NY, USA). The filtrate was diluted and injected into a chromatography system (Dionex 2500, Dionex Co., New York, NY, USA). For sugar analysis, the solvent was 18 mM NaOH at a flow of 1 mL min^{-1}, CarboPac PA10 (4 × 250 mm; Dionex Co.), and an amperometric detector with an Au electrode was used. Fructose and glucose content were calculated by using external standards. The same extract used in the sugar analysis was used in organic acid analysis. IonPac ICE-AS6 column (9 × 250 mm; Dionex Co.) was used for separation of acids and 0.4 mM heptafluorobutyric acid was used as an eluent at a flow rate of 1 mL min^{-1}. A suppressed detector, with an anion-

ICE micromembrane suppressor and 5 mN tetrabutylammonium hydroxide was used. External standard was used to calculate organic acid content.

Extraction and analysis of AA

The tomato fruit sample (2 g) was homogenized with 50 mL of buffer solution (4% metaphosphoric acid) and was filtered using an 8 μm cellulose of the filter paper (Whatman Intl. Ltd.). The mixture was filtered through a 0.45 μm polyvinylidene fluoride membrane of the syringe filter (Agilent Co.) and injected for L-ascorbic acid analysis into an HPLC system (Ultimate 3000, Dionex, Sunnyvale, CA, USA) under the following conditions. The mobile phase was acetonitrile and 50 mM $NH_4H_2PO_4$ (70:30, v/v), and the flow rate was 1.0 mL min^{-1}. The components were detected at 254 nm. A C18 reverse phase column (4.6 × 250 mm, 0.5 mm; Supelcosil TM C-18, Supelco, Bellefonte, PA, USA) was used for analysis (Kim et al., 2006).

Extraction and analysis of lycopene

Lycopene of tomato puree was extracted using solvent mixture of hexane/acetone/ethanol (2:1:1, v:v:v), and the lycopene concentrations in hexane layer were determined spectrophotometrically, according to the method described by Fish et al. (2002) with minor modifications.

Statistical analysis

Data were analyzed using SAS 9.1 version (SAS Inst. Inc., Cary, NC, USA) for Duncan's multiple range test (DMRT) at $P < 0.05$.

RESULTS AND DISCUSSION

Plant growth

Plant height was lowest in the treatment 9.8S among all the treatments and plant height in the S treatments at each EC level were lower than plant height in the treatments M and Lat each EC level, respectively (Table 2-2). Stem diameter, shoot fresh, dry weight and percent dry matter were affected by EC levels and container volumes (Bolarin et al., 1991; Cruz et al., 1990). Meanwhile, shoot fresh, dry weights, and percent dry matter in the treatments 9.8S, M and L could not be measured because that they were almost withered at harvest.

Plant height, stem diameter, shoot fresh, and dry weights measured at 92 days after transplanting decreased as EC level increased and root zone volume decreased. One of the explanations that has been suggested for the phenomenon of shoot growth reduction in response to root restriction is deficiency in other nutrients caused by root restriction (Bar-Tal et al., 1990; Hanson et al., 1987; Mutsaers, 1983). Plant growth is generally observed in plants exposed to salinity stress (Bolarin et al., 1991; Cruz et al., 1990; Mahajan and Tuteja, 2005; Munns, 2002; Munns et al., 2008). Especially, in the salinity treatment, the dry matter content of fruits increased because of reduced water uptake, which was predominantly due to reduced

Table 2-2. Plant height, stem diameter, shoot fresh, dry weight, and percent dry matter of tomato plant measured 92 days after transplanting as affected by EC levels and root zone volume.

EC (dS m^{-1})	Container volume	Plant height[z] (cm)	Stem diameter (mm)	Shoot fresh weight (g/plant)	Shoot dry weight (g/plant)	Percent dry matter
2.4	S	43.0 cd	10.2 cb	137.2 e	20.5 e	14.9 bc
	M	46.5 a	11.2 ab	295.3 b	37.6 b	12.7 d
	L	46.6 a	12.0 a	365.8 a	52.3 a	14.3 c
4.2	S	40.9 de	9.1 cde	130.4 e	19.7 e	15.1 abc
	M	44.9 abc	10.2 cb	244.5 c	34.1 bcd	14.0 cd
	L	45.6 ab	11.6 a	273.2 bc	34.7 bc	12.6 d
7.0	S	43.5 cb	8.7 de	107.6 e	17.8 e	16.5 a
	M	46.3 a	9.5 cd	210.3 d	31.3 cd	15.1 abc
	L	46.8 a	11.6 a	184.0 d	29.1 d	15.9 ab
9.8	S	39.9 e	6.6 g	-	-	-
	M	46.4 a	7.3 gf	-	-	-
	L	46.6 a	8.0 ef	-	-	-
Significance[y]						
EC (A)		*	***	***	***	***
Container volume (B)		***	***	***	***	***
A × B		NS	NS	***	***	*

Mean separation within columns by DMRT at $P = 0.05$.
[y]NS, *, ***Nonsignificant or significant at $P < 0.05$, or 0.001, respectively.

transpiration. These results agree with the improvement in fruit quality under saline conditions caused by the condensation of dry matter in fruits (Ehret and Ho, 1986; Ho et al., 1987). Root restriction and salt stress may be partly due to lower water potential (Azevedo-Neto et al., 2004; Jeffery and Sinclair, 1998). The decrease in the water potential occurred in both abiotic stresses results in reduced cell growth, root growth and shoot growth and also causes inhibition of cell expansion and reduction in cell wall synthesis (Chaitanya et al., 2003).

Fruit diameter and yield

Fruit diameter of tomatoes was the lowest in the treatment 9.8S, and the highest in the treatment 2.4L (Fig. 2-1). It decreased as EC level increased and root zone volume decreased. In the treatments of low EC with small pot size, the fruit diameter was not significantly different from that in the treatments of high EC with large pot size treatment (e.g., treatments 7.0L and 4.2M).Yield was highest in the treatment 2.4L and lowest in the treatment 9.8S (Fig. 2-2). In the treatments 2.4M, 4.2M, 7.0M, and 9.8M, yield decreased by 5.4, 31.9, 37.1, and 74.1%, respectively, compared to that in the treatment 2.4L. In the treatments 2.4S, 4.2S, 7.0S, and 9.8S, yield decreased by 19.8, 61.4, 63.8, and 83.4%, respectively, compared to that in the treatment 2.4L. Salt stress and root restriction reduced the fruit diameter and yield (Bar-Tal and Pressman, 1996; Estan et al., 2005). The effect of

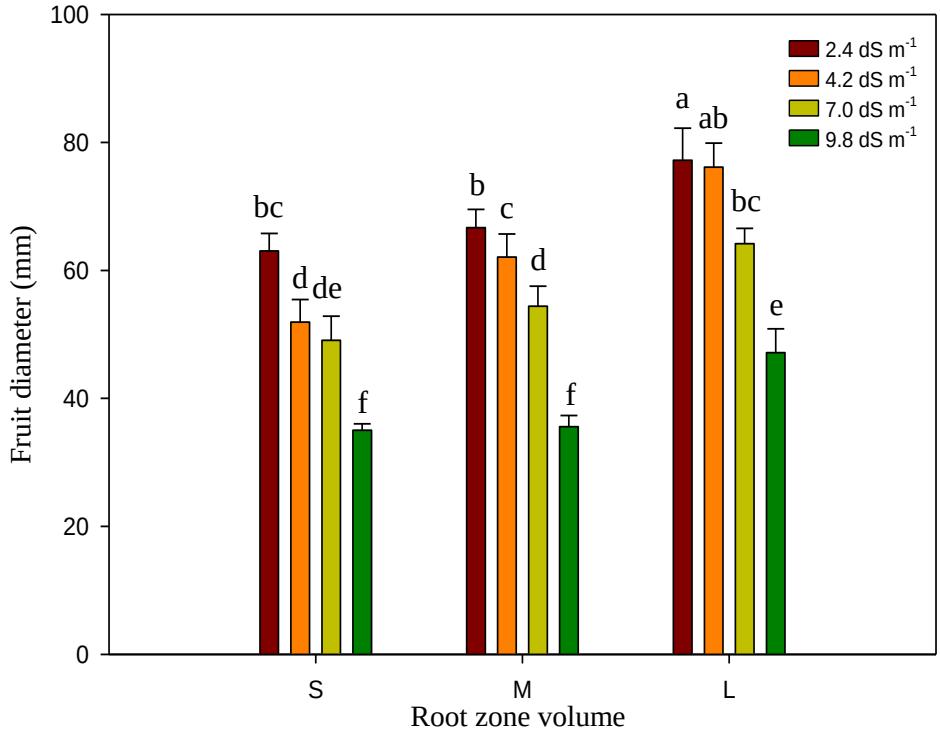

Fig. 2-1. Fruit diameter of tomato as affected by different EC and root zone volume treatments.

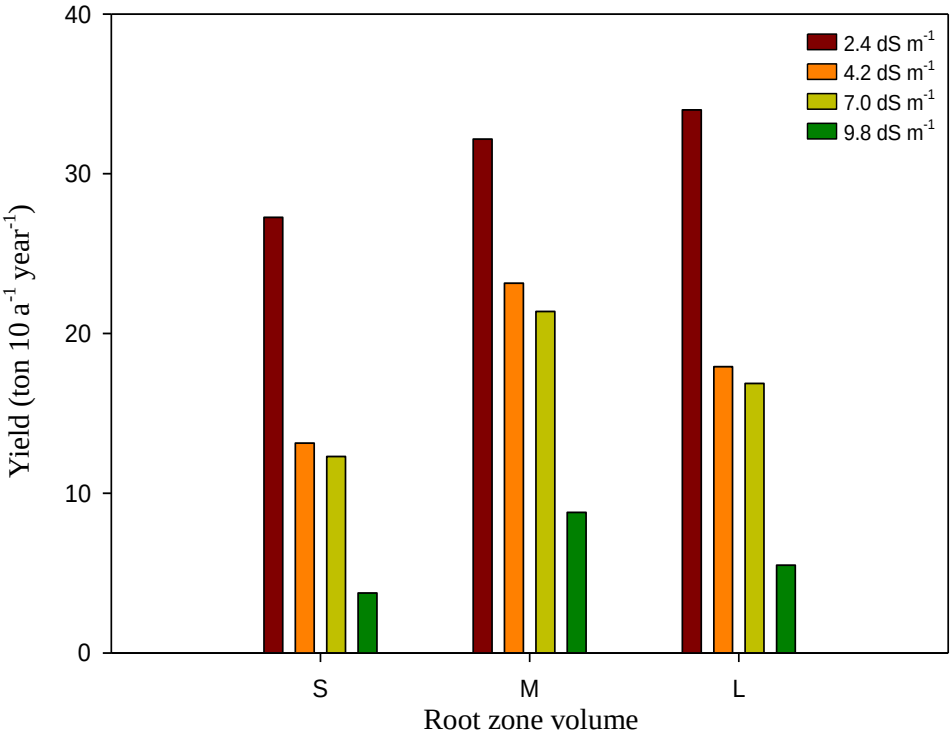

Fig. 2-2. Fruit yield of tomato plant as affected by different EC and root zone volume treatments.

root restriction on the total yield was greater than its effect on fruit number, because smaller fruits were produced by the root restricted plants (Bar-Tal et al., 1994, 1995). In Korea, the estimated annual production of tomato per 10a was 15 ton (MFAFF, 2008). The annual production of each treatment was not decreased except the treatments 9.8, because plant density increased as root zone volume decreased.

The major fruit physiological disorder observed in the previous studies on water stress was BER. However, the incidence of BER was not observed in all treatments in the present study. Root restriction reduced the incidence of BER as found previously (Bar-Tal et al., 1995). Three factors contributed to the reduction in the incidence of BER by root restriction. (1) Root restriction increased the number of fruits per leaf unit weight. The shift in the fruit/leaf ratio probably reduced the transpiration through leaves relative to fruits (a high correlation between leaf weight and leaf area in greenhouse tomato, independent of root restriction, has been reported by Bar-Tal et al., 1995). Since the ratio between the water supply to the fruits from the leaves and that directly from the roots via the xylem is the dominant factor controlling the Ca supply to the fruits (Ehret and Ho, 1986; Ho et al., 1987; Wiersum, 1966), it can be considered that increased fruit number per leaf area could contribute to an increased Ca supply to the developing fruits and to reduced incidence of BER. (2) Root restriction reduced the growth rate of the fruits. The second factor to affect the incidence of BER and fruit growth

was reported by Ho et al. (1993). (3) The third factor that may affects the incidence of BER, the K/Ca ratio in the plant organs. Root restriction reduced the K/Ca concentration ratio in the plants as Bar-Tal and Pressman (1996) had reported.

Sugars and organic acids at each stage

In all the treatments, contents of glucose, fructose and malic acid in tomato fruits were not significantly different at green and break (GB) stages of tomato fruits (Table 2-3). In the treatments 7.0S, 7.0M, and 7.0L citric acid was increased compared other treatments at the GB stages. In the treatments 4.2S, 4.2L, and 7.0S glucose and fructose in tomato fruits were higher than other treatments at the turning and pink (TP) stages (Table 2-4). Glucose content in tomato fruits was affected by EC levels and root zone restriction, and fructose in tomato fruits was affected by root zone restriction only at the TP stages. Contents of malic and citric acid in tomato fruits increased in the treatments 7.0S, 7.0M, and 7.0L compared with other treatments at the TP stages. Content of organic acids were mainly affected by EC levels at the TP stages. Contents of glucose, fructose, and citric acid in tomato fruits increased in the treatments S compared with the treatments M and L at each EC level of the light red and red (LR) stages (Table 2-5). Contents of glucose, fructose, and citric acid in tomato fruit increased as EC levels increased and root zone volume decreased at the LR stages.

Table 2-3. Contents of glucose, fructose, malic acid, and citric acid of tomato fruits as affected by different EC and root zone volume treatments at the green and break (GB) stages.

EC (dS m^{-1})	Container volume	Glucose (mg g^{-1} FW)	Fructose (mg g^{-1} FW)	Malic acid (mg g^{-1} FW)	Citric acid (mg g^{-1} FW)
2.4	S	24.16 b	26.75 a	30.07 ab	88.24 bc
	M	34.73 ab	36.65 a	23.79 b	63.73 c
	L	28.01 ab	29.58 a	30.06 ab	84.51 bc
4.2	S	29.50 ab	32.75 a	35.11 ab	101.66 bc
	M	28.85 ab	29.45 a	26.64 b	67.07 c
	L	38.03 a	38.55 a	31.28 ab	85.87 bc
7.0	S	35.68 ab	38.31 a	41.47 ab	134.97 ab
	M	30.09 ab	29.98 a	46.56 a	162.28 a
	L	29.42 ab	32.31 a	41.67 ab	118.76 abc
Significance[y]					
EC (A)		NS	NS	*	**
Container volume (B)		NS	NS	NS	NS
A × B		NS	NS	NS	NS

[z]Mean separation within columns by DMRT at $P = 0.05$.
[y]NS, *, **Nonsignificant or significant at $P < 0.05$, or 0.01, respectively.

Table 2-4. Contents of glucose, fructose, malic acid, and citric acid of tomato fruits as affected by different EC and root zone volume treatments at the turning and pink (TP) stages.

EC (dS m^{-1})	Container volume	Glucose (mg g^{-1} FW)	Fructose (mg g^{-1} FW)	Malic acid (mg g^{-1} FW)	Citric acid (mg g^{-1} FW)
2.4	S	39.05 bc	42.56 abc	28.65 b	113.97 c
	M	35.36 c	39.20 bc	22.58 b	94.35 c
	L	38.55 bc	39.99 bc	25.07 b	108.60 c
4.2	S	50.35 a	49.70 a	14.36 b	127.33 bc
	M	36.35 c	37.39 c	19.84 b	115.55 c
	L	44.59 ab	44.74 abc	24.32 b	118.84 c
7.0	S	46.46 ab	47.39 ab	46.85 a	166.49 a
	M	38.56 bc	41.61 abc	48.62 a	169.29 a
	L	39.79 bc	40.93 bc	42.52 a	158.25 ab
Significance[y]					
EC (A)		*	NS	***	***
Container volume (B)		**	*	NS	NS
A × B		NS	NS	NS	NS

[z]Mean separation within columns by DMRT at $P = 0.05$.
[y]NS, *, **, ***Nonsignificant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

Table 2-5. Contents of glucose, fructose, malic acid, and citric acid of tomato fruits as affected by different EC and root zone volume treatments at the light red and red (LR) stages.

EC ($dS\ m^{-1}$)	Container volume	Glucose ($mg\ g^{-1}$ FW)	Fructose ($mg\ g^{-1}$ FW)	Malic acid ($mg\ g^{-1}$ FW)	Citric acid ($mg\ g^{-1}$ FW)
2.4	S	49.03 abc	51.24 abc	11.88 abc	112.72 cd
	M	33.39 d	36.96 d	9.88 bc	89.17 e
	L	38.80 cd	41.45 cd	11.44 abc	95.17 de
4.2	S	52.49 ab	54.48 ab	10.21 bc	120.19 c
	M	43.14 bcd	43.98 cd	16.30 a	114.39 cd
	L	40.64 cd	42.24 cd	9.18 c	92.55 e
7.0	S	54.29 a	56.97 a	15.42 ab	165.46 a
	M	46.65 abc	48.11 abc	16.96 a	142.01 b
	L	44.72 abc	46.18 bcd	11.78 abc	130.86 bc
Significance[y]					
EC (A)		*	*	NS	***
Container volume (B)		***	***	NS	***
A × B		NS	NS	NS	NS

[z]Mean separation within columns by DMRT at $P = 0.05$.
[y]NS, *, ***Nonsignificant or significant at $P < 0.05$, or 0.001, respectively.

In all the treatments malic acid in tomato fruits was not significantly different at LR stages. Sucrose content in tomato fruits was not significantly different among all the treatments (data not shown). Sucrose is detected in trace amounts in tomato fruits (Davies and Kempton, 1975; Haila et al., 1992). The higher content of sugar in the fruits as a result of active phloem unloading under saline conditions plays an important role in osmotic adjustment and acts as a compatible solute (Greenway and Munns, 1980; Muuns et al., 1982) similar to proline (Delauney and Verma, 1993) and betaine (Hanson and Wyse, 1982).

Total soluble solids content, titratable acidity, sugar to acid ratio

TSS remarkably increased in the treatments 4.2S, 7.0S, and 9.8S and TA remarkably increased in the treatments 7.0S and 9.8S compared to those in other treatments including the treatment 2.4S that had same degree of root zone restriction (Fig. 2-3). Sugar-acid ratio in the treatments 2.4S was the lowest and sugar-acid ratio in the treatment 4.2S was higher than that in the treatments 7.0S and 9.8S. TSS and TA showed similar trend to sugars and organic acids in tomato fruits. These results show that the increase in TSS and TA in fruits with salinity treatment and root zone restriction was the result of not only condensation due to reduced water uptake, but also the active import to the fruits from increasing hydrolysis reactions of sugars and organic acids (Saito et al., 1996). Results show that tomato fruit quality

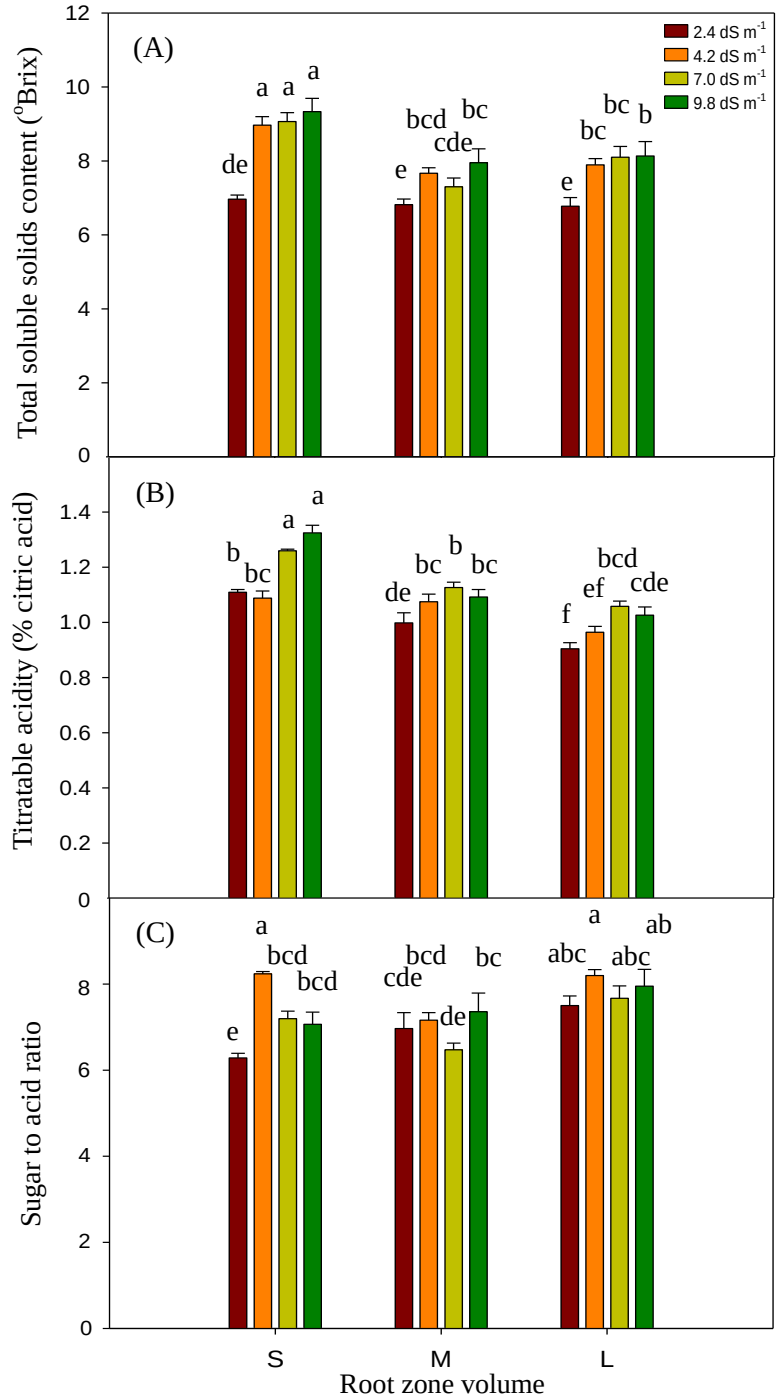

Fig. 2-3. Contents of total soluble solids (A), titratable acidity (B), and sugar to acid ratio (C) of tomato fruits as affected by different EC and root zone volume treatments.

could be much improved with minimized yield reduction if the salt stress and root zone restriction treatments are effectively combined e.g., the treatment 4.2S.

Ascorbic acid and lycopene content

AA content in tomato fruits increased as EC levels increased at the treatments M and L among the all ripeness stages (Fig. 2-4). Meanwhile, AA in tomato fruits was not significantly different at the treatments S among the all ripeness stages.

Lycopene content in tomato fruits was increased during fruit ripening as well known (Fig. 2-5; Giovanelli, 1999; Jimenez et al., 2002; MacDonald and Rajasekaran,2009). In the treatment 4.2S lycopene content was highest among the all the treatments at TP and LR stages. A tomato fruit is known as a climacteric fruit and the fruit ripening process begins with an increase in respiration and ethylene synthesis. Therefore, slowing or inhibition of tomato fruit ripening was observed in ethylene-suppressed transgenic plants (Oeller et al., 1991; Theologis et al., 1993). Application of exogenous ethylene triggers ripening tomato fruits and the technique has been applied commercially to green-harvested tomatoes from the open field. Development of red color (primarily attributed to the presence of lycopene) in fruits begins after this initial increase in ethylene. The lycopene concentration increases rapidly during this maturation process. High EC in

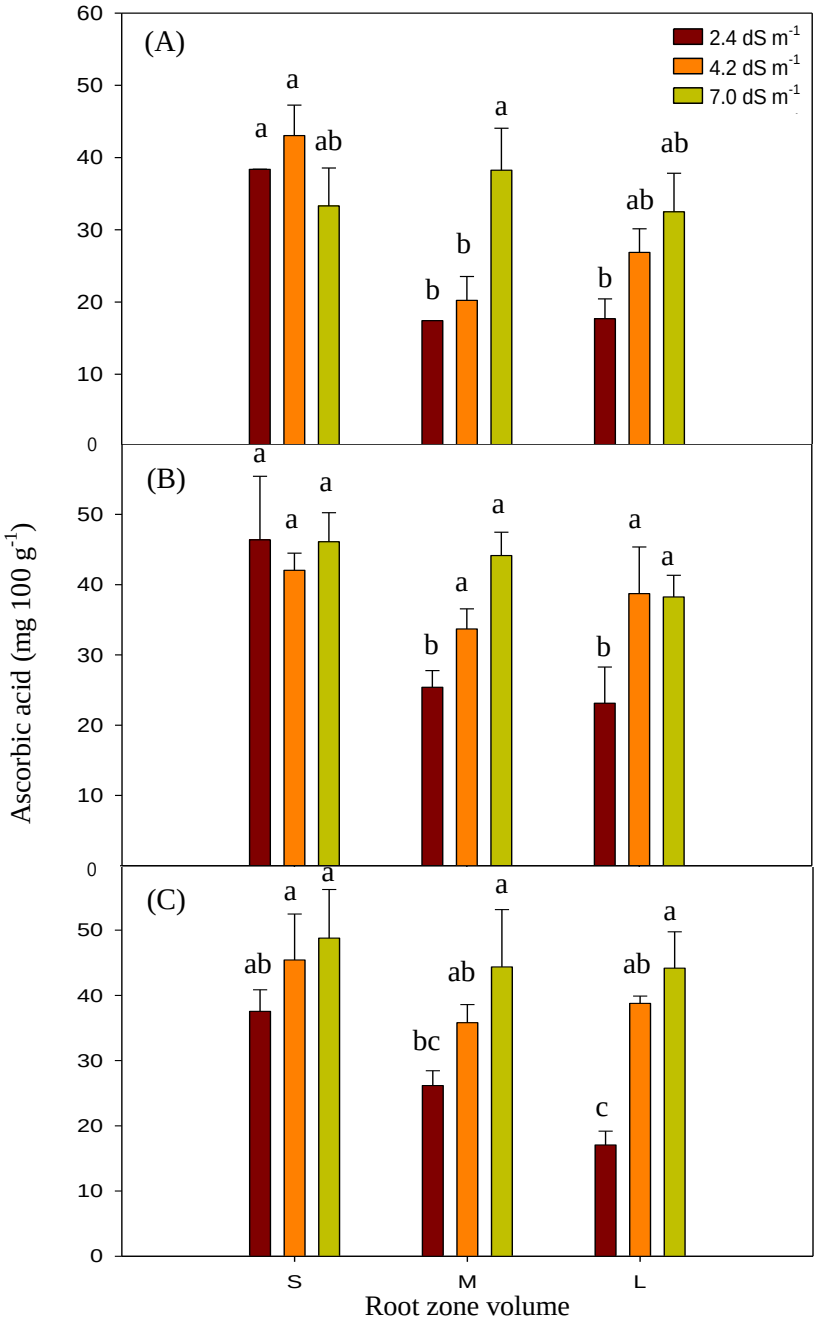

Fig. 2-4. Ascorbic acid content in fruits of tomatoes as affected by different EC and root zone volume treatments at the green and break (GB, A), the turning and pink (TP, B), and the light red and red (LR, C) stages

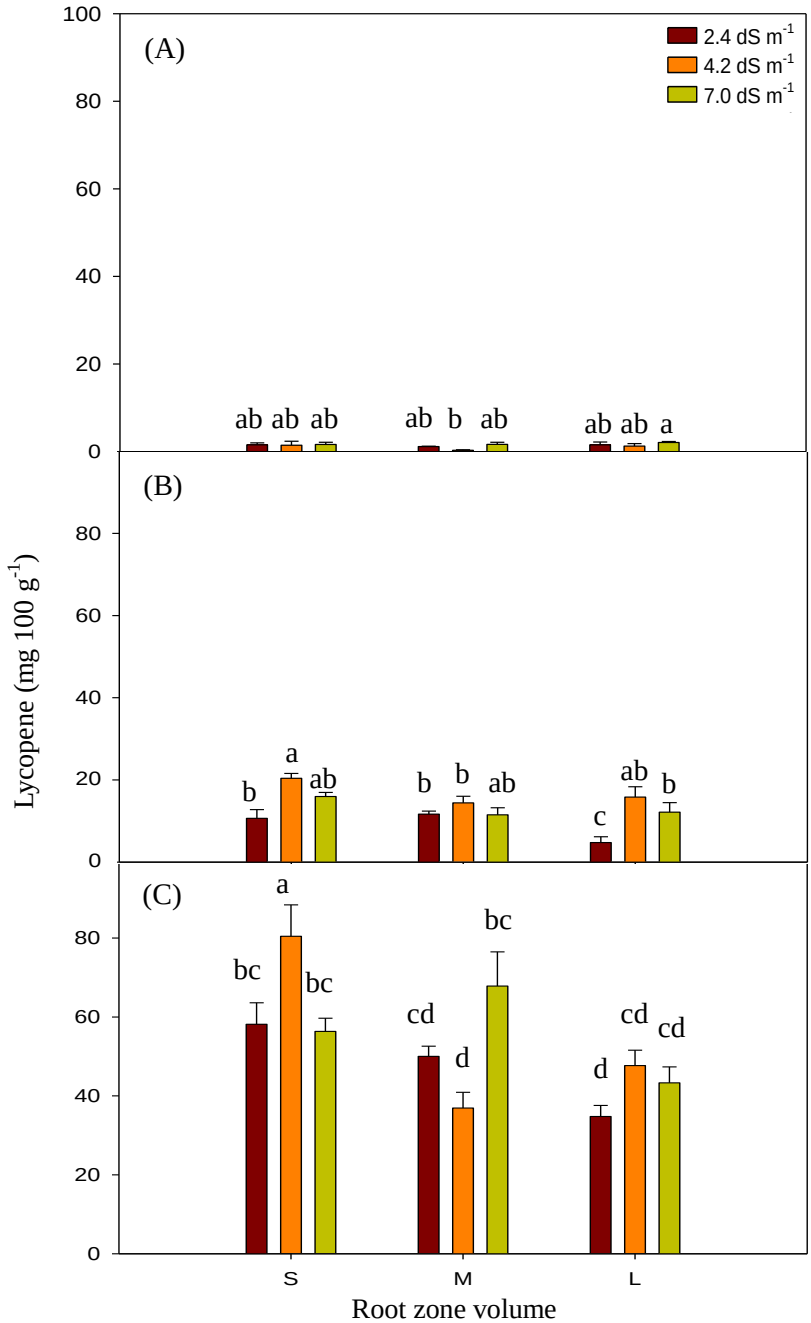

Fig. 2-5. Lycopene content of tomato fruits as affected by different EC and root zone volume treatments at the green and break (GB, A), the turning and pink (TP, B), and the light red and red (LR, C) stages.

the nutrient solution and thereby high EC nutrient solution reportedly induced ethylene emission across several tomato cultivars (Mizrahi, 1982). Botella et al. (2000) showed that irrigation of tomato plants with 40–60 mM NaCl increases red color, ethylene production and total ACC (1-aminocyclopropane-1-carboxylic acid). This suggests that ethylene production induced by osmotic and/or salt stress enhanced the concentration of lycopene in the tomatoes. Thus, under water stress, tomatoes mature earlier and accumulate more lycopene during the time before harvest. Wu and Kubota (2008) showed that the light compensation point of tomato leaves was increased under high EC treatment, which means that tomato plants had a higher respiration rate under high EC treatment. This may suggest that overall enhancement of metabolism under high EC treatment resulted in the increased ethylene production and lycopene in the fruit ripening process. Although the exact biological mechanisms that contribute to the enhancement of lycopene concentrations under high EC stress are not known, the above evidence suggests that ethylene synthesis triggered by osmotic and/or salt stress could be central to the increase in lycopene deposition within the tomato fruits.

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CONCLUSIONS

Total soluble solids (TSS) content in tomato fruits increased as EC levels of nutrient solution increased and the effect of increased TSS was greater when the nutrient solution with higher EC levels was applied after transplanting, while growth of tomato plants was inhibited and yield of tomatoes decreased in summer and winter seasons. Tomato fruit quality including TSS, titratable acidity, ascorbic acid, and lycopene contents increased by the combination of high EC level and small container volume. Incidence of BER could be avoided by lowering the EC level with smaller container volume without deterioration of fruit quality in cool season. Improvement of production per unit area caused by increasing plant density with small container volume could be regarded to make up for reduction of yield per plant. In the present study, results show that increasing EC of nutrient solution and decreasing root zone volume after transplanting can be a feasible option for producing high-quality tomato in high-density and low-truss cultivation system.

ABSTRACT IN KOREAN

저단 밀식 재배 시 토마토에 수분 스트레스를 적용함으로써 과실의 당도를 높이는 적절한 방법을 구명하고자 본 연구를 수행하였다. 제1장에서는 토마토의 생육 단계별로 배양액의 EC를 최대 13.7dS m^{-1} 까지 높였을 경우 과실의 당도 변화와 염 스트레스의 정도, 재배 계절이 토마토 배꼽썩음과 발생에 미치는 영향을 구명하였다. 양액의 EC가 증가함에 따라 토마토 식물체 생육은 저해되고 수확량은 감소하는 반면, 과실의 당도는 증가하였다. 토마토 과실의 당도는 겨울 재배보다 여름 재배 시에 더욱 높았고 겨울 재배 토마토에서 훨씬 적은 수준의 배꼽썩음과 발생률을 보였다. 제2장에서는 높은 EC로 인해 나타나는 생육 저해, 수확량 감소, 높은 배꼽썩음과 발생률과 같은 부정적인 효과를 줄이기 위해 염 스트레스와 근권부 제한 처리를 조합한 실험을 진행하여 과실의 품질을 조사하였다. 토마토 식물체의 생장 및 과실의 수확량은 배양액 EC가 증가할수록, 재배 포트 크기가 작을수록 감소하였다. 과실의 당도, 산도, 그리고 비타민 C는 포트 크기가 작을수록 증가하였다. 당도와 산도는 각각 포트크기 S에서 4.2dS m^{-1} 이상의 EC로 재배한 처리구, 7.0dS m^{-1} 이상의 EC로 재배한 처리구에서 현저히 높았다. 과당, 포도당, 구연산 및 비타민 C는 과실이 성숙할수록 EC와 근권부 제한에 의한 결과가 명확하게 나타났다. 리코펜 함량은 4.2S 처리구에서 가장 높았다. 본 결과를 통해, 토마토 재배 시 양액의

EC를 생육 초기 단계에 높이면, 양액의 EC 증가로 인한 토마토 과실의 당도 증진에 더욱 효과적이라는 것을 확인할 수 있었다. 또한, 배양액의 EC와 근권 제한 처리를 조합함으로써 수확량 감소와 배꼽썩음과 발생을 최소화시키면서 과실 품질을 향상시킬 수 있음을 확인하였다. 토마토 저단 밀식 재배 시에는 약 200mL 정도의 포트에서 EC 4.2dS m^{-1}로 재배하는 것이 적합한 것으로 판단하였다.

주요어: high density-low truss cultivation, root zone restriction, salt stress, total soluble solids content, water stress

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