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Effects of Salinity Treatment Duration and Planting Density on Size and Sugar Content of Hydroponically Grown Tomato Fruits

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Tomato plants were grown using a nutrient film technique in a hydroponic system to evaluate the effects of starting time and duration of salinity treatment and the interaction between salinity and planting density on fruit yield and quality. The electrical conductivity (EC) of the nutrient solution was maintained until the anthesis of plants. Sodium chloride was added to the standard nutrient solution until EC 8.0 $dS \cdot m^{-1}$ and this level was maintained from the anthesis of the first flower truss until the fruit harvest (whole treatment); 8.0 $dS \cdot m^{-1}$ solution was applied from anthesis of the first truss until 20 days after anthesis (DAA) (early treatment) and from 20 DAA until the fruit harvest (late treatment). The average fruit weights in the whole, early, and late treatments were 46, 71, and 58% of the control weight, respectively. Fruit radius and cell size were also reduced under each salinity treatment; however, the estimated number of cells per fruit was not significantly affected by the salinity treatment. The levels of total soluble solids (Brix%) were 6.2 in the control and 9.9, 7.7, and 9.1 in the whole, early, and late treatments, respectively. Incidences of blossom-end rot were 0, 33, 25, and 16% in the control, whole, early, and late treatments, respectively. The influence of planting density (8.5–9.5 plants/m^2) under saline conditions on fruit size and sugar content was not considerable unremarkable. The fruit yield at high planting density increased more than that at a low plating density under salinity treatment.

Key Words: fruit size, planting density, salinity, sugar content, tomato.

Introduction

Sugar content is considered to be one of the most important factors in tomato fruit quality and consumer satisfaction (Malundo et al., 1995). Consumers require tomatoes high in sugar content, with a Brix value greater than 8.0%, which is considered high quality. Salinity stress in the root zone is known to improve tomato fruit quality in terms of the content and composition of soluble sugars and acids (Adams, 1991; Adams and Ho, 1989; Cuartero and Fernandez-Munoz, 1999; Ehret and Ho, 1986). However, salinity stress is accompanied by yield loss through a reduction in fruit weight, but not in the number of fruits (Li et al., 2001; Willumsen et al., 1996). Water influx into fruits is reduced by the high osmotic pressure of the irrigation solution, and this water stress inhibits fruit size (Bolarin et al., 2001; Chretien et al., 2000; Ehret and Ho, 1986; Li et al., 2001; Mavrogianopoulos et al., 2002). The duration of salinity stress is important because it affects fruit yield and quality. However, there have been few studies on the starting time and duration of salinity treatment in the tomato (Sakamoto et al., 1999).

High salinity stress also increases the incidence of blossom-end rot (BER), a physiological disorder caused by a local lack of calcium in the fruit (Adams and Ho, 1992; Chretien et al., 2000; Ehret and Ho, 1986; Franco et al., 1994; Willumsen et al., 1996) because of decreased Ca^{2+} uptake by the roots and Ca^{2+} transport via the xylem to the fruit under high salinity stress (Belda and Ho, 1993; Belda et al., 1996; Ho et al., 1993).

The cropping system of low node-order pinching that enables culturing 4–5 times per year is suitable for the production of tomatoes with a high sugar content because it is relatively easier to control plant growth under this system than under the common multi truss cropping system. However, a high planting density is required to increase productivity (yield per area) in this system, particularly under saline conditions. This could compensate for reductions in fruit size and increase fruit yield. However, there have also been few reports on the effects of salinity treatment on fruit yield and quality in such cropping systems. The purpose of the present study was to investigate the effects of the starting time and duration of salinity treatment and interaction between salinity stress and planting density on fruit yield, size, and quality using a low node-order pinching and dense planting culture system.

Materials and Methods

Plant materials and growing conditions

Tomato (*Lycopersicon esculentum*, Mill. 'House

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Momotaro', Takii & Co., Ltd., Japan) seeds were sown in trays with moist vermiculite in a greenhouse. When the cotyledons were fully open, the seedlings were transplanted into rockwool cubes (125 cm^3, Nittobo Co., Ltd., Japan) and grown in a deep flow technique (DFT) system with Otsuka-A nutrient solution (Otsuka Chemical Co., Ltd., Osaka, Japan) adjusted to an electrical conductivity (EC) of 1.2 $dS \cdot m^{-1}$ and pH of 6.5–7.0. After 2 weeks, the seedlings were transplanted to a nutrient film technique (NFT) system in a greenhouse. When the first flower opened on the first truss of each plant, pollination was promoted by a vibrator and spraying of 2-methyl-4-chlorophenoxyacetic acid (4-CPA). All lateral shoots were removed as they appeared and the plants were pinched above the second truss with two true leaves over the truss, and extra fruits other than 4 fruits per truss were pruned. Otsuka-B nutrient solution (Otsuka Chemical Co., Ltd.), adjusted to an EC of 2.5 $dS \cdot m^{-1}$ and pH of 6.5–7.0, containing NH_4^+, 20; NO_3^{3-}, 210; PO_4^{3-}, 93; K^+, 377; Ca^{2+}, 219; Mg^{2+}, 80; Mn^{2+}, 1.0; B^-, 1.0; Fe^{3+}, 2.9; Cu^{2+}, 0.02; Zn^{2+}, 0.04; and Mo^+, 0.02 ppm was supplied in all experiments. During winter, the greenhouse air temperature was maintained above 10°C by heating.

Salinity treatments

In the control treatments, the EC level was 0.8 $dS \cdot m^{-1}$ at transplant and gradually increased to 2.5 $dS \cdot m^{-1}$ at harvest. For the salinity treatments, NaCl (approximately 3 $g \cdot L^{-1}$) was added to the standard nutrient solution to obtain an EC of 8.0 $dS \cdot m^{-1}$.

Effects of salinity treatment start time and duration on fruit development

The experiment was carried out twice from 13 March to 6 July 2003 (Exp. 1) and from 28 August 2003 to 30 January 2004 (Exp. 2). Three salinity treatments with different start times and durations (i.e., whole, early, and late treatments) were investigated. In the whole treatment, salinity treatment began from the anthesis of the first truss and was continued until the end of harvesting. In the early and late treatments, high salinity levels were maintained from the anthesis of the first truss to 20 days after anthesis (DAA) and from 20 DAA to the end of the harvesting, respectively (Fig. 1). The planting density in each treatment was 2.2 plants/m^2, with 150 cm ridge widths and 30-cm plant spacing. Twenty plants per treatment were transplanted, and under each treatment, an average of a hundred fruits was analyzed for the fresh weight and total soluble solids.

Planting density under high salinity condition

Tomato plants were grown from 28 August 2003 to 30 January 2004. Whole salinity treatments were applied as described above. Three planting densities were arranged: low, 6.7 plants/m^2 (ridge widths of 150 cm and 10-cm plant spacing); medium, 8.3 plants/m^2 (ridge widths of 150 cm and 8-cm plant spacing); and high, 9.5 plants/m^2 (ridge widths of 150 cm and 7-cm plant spacing). For each density, twenty plants per treatment were transplanted, and for each treatment, an average of a hundred fruits was analyzed for the fresh weight and total soluble solids.

Fruit yield and quality analysis

Fruits were harvested separately by truss. The total weight and number of fruits were recorded. Fruits were classified as marketable or unmarketable, the latter characterized by BER, cracking, deformity, or small size (< 40 g fresh weight).

The total soluble solid content of fruits was determined using a hand refractometer (N-20E, Atago Co., Ltd., Tokyo, Japan). The titratable acidity of fruits was examined by the titrate method. A 10-gram fruit was homogenized and centrifuged for 10 min at 10000 rpm. The supernatant was then passed through filter paper and filtrate was diluted with a 5-times dilution of distilled water. It was then titrated with 0.1 N NaOH. Titratable acidity was expressed as the citric acid concentration.

Sugar content and composition were determined using high performance liquid chromatography (HPLC). Fresh fruits were frozen in liquid nitrogen and kept at –80°C until analyzed for sugar content. A 10-gram fruit was homogenized and centrifuged for 10 min at 10000 rpm.

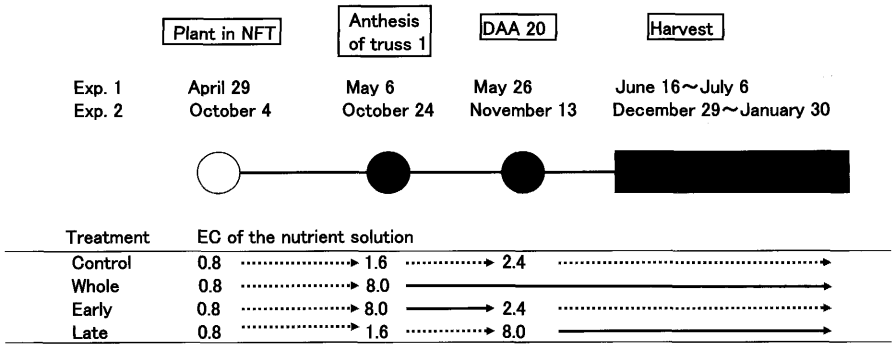

Fig. 1. Time schedules of salinity stress in tomato plants cultivated by the NFT system. EC of the nutrient solution was increased by adding NaCl to the standard nutrient solution.

The supernatant was filtered through filter paper and a 0.45-μm Millipore filter and injected into an HPLC system equipped with TSK-GEL AMIDE-80 (4.6 mm i.d. × 250 mm; TOSOH Co., Tokyo, Japan) and an RI detector. The measurement was performed at 80°C using acetonitrile:water 75 : 25 (v/v) as the mobile phase at a flow rate of 1 mL·min^{-1}.

Histological analysis of fruits

We observed cells and tissues of tomato fruits grown under salinity treatments according to a method modified from Higashi et al. (1999). Harvested fruits of the first truss of each treatment (at 43 DAA) were cut on the equatorial plane and the radius was measured. Parts of the fruits were then cut and fixed in FAA solution (50% ethanol, 10% formaldehyde, and 5% acetic acid (v/v)). Fixed tissues were dehydrated through a graded butanol series and embedded in paraffin (Paraplast Plus, Kendall). Horizontal sections were cut using a microtome and collected on glass slides. After incubation on hot plates at 50°C, sections were stained with 0.05% toluidine blue and observed under an optical microscope. Cell size was determined as follows: the number of cells in a 1-mm scale was counted, and 1 mm was then divided by the cell count. The estimated number of cells per fruit was determined on the basis of the fruit radius and cell size.

Results

Effects of salinity treatment duration on fruit development (Exp. 1)

All salinity treatments reduced the fruit fresh weight by 30–54% versus the control. Although the fruit fresh weight was 111.8 g/fruit in the control group, the fruit fresh weights in the whole, early, and late treatments were 51.5, 78.8, and 64.8 g/fruit, respectively (Fig. 2). However, the number of fruits per plant was 6–7 for all treatments; thus, the salinity level did not significantly affect the fruit number per plant.

The frequency of BER fruits increased with salinity treatment. In the whole and early treatments, 33.1 and 25.4% of the fruits showed BER, respectively. However, only 16.0% of the fruits in the late treatment showed BER. Conversely, the incidence of cracking fruit was reduced by the salinity treatment, compared with control fruits. In the whole, early, and late treatments, 8, 60, and 13% of the fruits showed cracking, respectively.

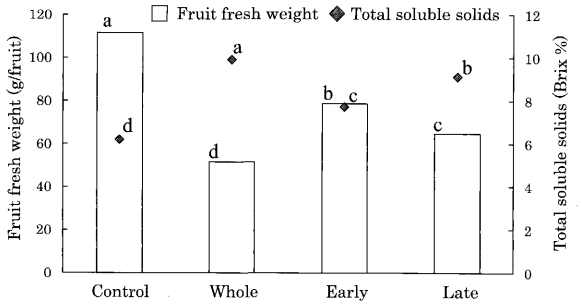

Fig. 2. Effect of starting time and duration of salinity treatment on fruit fresh weight and total soluble solids in fruits (Exp. 1). Different letters indicate significant differences by Fisher's PLSD test, $P=0.05$.

The average fresh weights of fruits were decreased by salinity treatments (Fig. 2). In contrast, the salinity treatment increased the Brix of the fruit. The Brix was 6.2% in the control, but 9.9, 7.7, and 9.1% in the whole, early, and late treatments, respectively (Fig. 2). The titratable acidity of the fruit was significantly higher in the whole and late treatments than that in the control (Table 1). Fructose, glucose, and sucrose contents were also significantly higher in the salinity treatments than those in the control. The glucose and total sugar contents in both the whole and late treatments were higher than those in the early treatment and control.

The radii of fruits in the whole and late treatments were 79% and 90% of that in control fruits (Table 2). The average cell sizes in the whole and late treatments were 65 and 79% of that in the control. However, the estimated number of cells per fruit was not significantly affected by salinity treatment.

Effects of planting density under high salinity condition on fruit fresh weight and quality (Exp. 2)

Under different planting densities without salinity treatment, the fruit fresh weight was reduced more in the medium and high planting densities than in the low one (Fig. 3A). The fruit fresh weights in the medium- and high-planting densities were reduced by almost 15%

Table 1. Effect of starting time and duration of salinity treatment on titratable acidity and sugar content of tomato fruit (Exp. 1).

Treatment	Titratable acidity[z] (mg per 100 gFW)	Sugar contents (mg per gFW)			
		Fructose	Glucose	Sucrose	Total[y]
Control	0.24 b[x]	19.8 b	21.1 c	0.44 b	41.3 c
Whole	0.38 a	21.7 a	24.3 a	1.85 a	47.9 a
Early	0.27 b	19.4 b	21.3 c	1.14 ab	41.8 c
Late	0.35 a	20.5 b	22.7 b	0.57 b	43.8 b

[z] Expressed as citric acid.
[y] Sum of fructose, glucose and sucrose contents.
[x] Different letters within a column indicate significant differences by Fisher's PLSD test, $P=0.05$.

Table 2. Effect of starting time and duration of salinity treatment on cell size and estimated number of cells in tomato fruits (Exp. 1).

Treatment	Fruit radius (mm) (A)	Cell size (μm) (B)	Estimated number of cells (A/B)
Control	30.9 a[z]	385.8 a	80.1 a
Whole	24.6 b	254.2 b	96.8 a
Early	29.0 ab	323.7 ab	89.2 a
Late	27.7 ab	304.1 ab	91.1 a

[z] Different letters within a column indicate significant differences by Fisher's PLSD test, $P=0.05$.

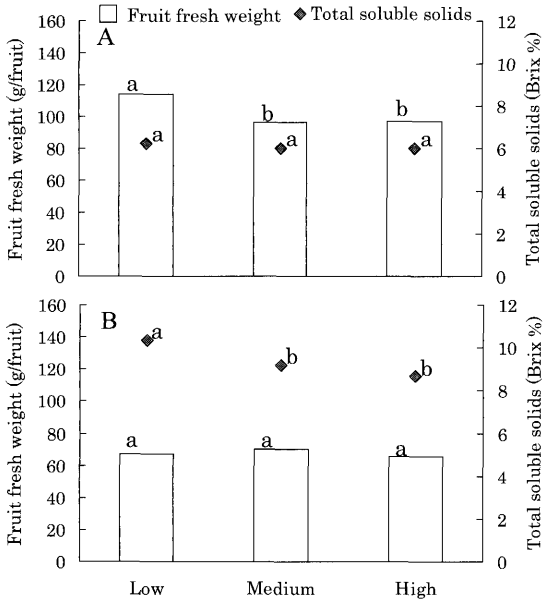

Fig. 3. Effect of salinity treatment and planting density on fruit fresh weight and total soluble solids in tomato fruits (Exp. 2). A, No salinity treatment; B, Whole treatment. Different letters indicate significant differences by Fisher's PLSD test, $P=0.05$.

of that in the low-planting density.

In the whole treatment with salinity, the yields of all planting densities were lower than those without salinity treatment. Fruit fresh weight was not affected by planting density in the whole treatment with salinity (Fig. 3B).

The percentage of total soluble solids with the salinity treatments was higher than those without salinity treatment. The total soluble solids were reduced with increasing planting density under salinity treatment (Fig. 3B). Medium- and high-density plantings showed 11 and 16% losses in soluble solids, respectively, than the low-density plantings. In contrast, total soluble solids without salinity treatment were not affected by planting density (Fig. 3A). Sugar contents were affected by salinity treatment. However, the effect of planting density on the sugar content was not clear (Table 3).

Titratable acidity and sugar content were significantly increased by salinity treatment, but were not affected by planting density either in the presence or absence of salinity treatment (Table 3).

Discussion

In the whole treatment, the fruits were exposed to high salinity conditions for 40 to 60 days, and this promoted sugar accumulation in the fruit compared to both the

Table 3. Effect of salinity treatment and planting density on titratable acidity and sugar contents (Exp. 2).

Treatment		Titratable acidity[z] (mg per 100 gFW)	Sugar contents (mg per gFW)			
Salinity[v]	Planting density[w]		Fructose	Glucose	Sucrose	Total[y]
Control	Low	0.22	17.8	18.9	0.51	37.2
	Medium	0.22	16.2	16.1	0.44	32.7
	High	0.23	18.8	19.0	0.78	38.6
Whole	Low	0.36	23.1	25.2	2.09	50.4
	Medium	0.37	22.2	24.1	1.31	47.6
	High	0.35	21.8	23.6	2.51	47.9
Significance[x]						
Salinity		***	***	***	**	***
Planting density		NS	NS	NS	NS	NS
Salinity × Planting density		NS	NS	NS	NS	NS

[z] Expressed as citric acid.
[y] Sum of fructose, glucose and sucrose contents.
[x] NS, *, **, *** denote nonsignificant or significant differences at $P=0.05$, 0.01, or 0.001, respectively by the 2-way ANOVA test.
[w] Planting density: Low, 6.7 plants/m^2; Medium, 8.3 plants/m^2; High, 9.5 plants/m^2.
[v] Salinity: Control, the EC level was 0.8 dS·m^{-1} at planting, then it was gradually increased to 2.5 dS·m^{-1} at harvest. Whole, salinity treatment (EC level was 8.0 dS·m^{-1}) was started from the anthesis of the first truss until harvest.

early and late treatments. However, the salinity treatment during the early developmental stages of the fruit had less of an effect than that during the late developmental stages (Fig. 2, Table 1). This suggests that the accumulated sugar is diluted by a rapid influx of water into the fruits by the end of the early salinity treatment. Therefore, the effects of salinity on the sugar content of the fruit depended on the starting time and duration of the salinity treatment. Hence, in terms of sugar accumulation, salinity treatment was more effective in the later stage of fruit development than in the early stage.

Salinity stress improved tomato fruit quality in terms of higher concentrations of soluble sugars and acids, but this was accompanied by yield loss, mainly due to reduced fruit fresh weights (Fig. 3). Ehret and Ho (1986), Ho et al. (1987) and Sakamoto et al. (1999) reported that salinity-related improvement in fruit quality, i.e., a high concentration of quality constituents, may occur because salinity stress could considerably inhibit water uptake by roots and result in decreased water influx into the tomato fruit.

Cuartero and Fernandez-Munoz (1999) reported that fruits from salt-treated plants seem to grow normally during the cell division phase; however, during the cell expansion phase, the deleterious effects of salt were observed. In this experiment, while the estimated number of cells per fruit was not affected by salinity, the cell size was reduced by the salinity treatment and showed a trend similar to that of fruit fresh weight (Table 2). These results suggest that reduced fruit size under salinity treatment was a result of the suppression of cell enlargement caused by the reduced influx of water into fruit cells. This suggestion is supported by the results of Cuartero and Fernandez-Munoz (1999) that fruit reduction in the cell expansion phase is the consequence of a reduction in the water content of fruit. In the early treatment, the inhibition of fruit enlargement was less than that in the whole treatment. The inhibition of cell enlargement by salinity treatment recovered with the application of the standard nutrient solution following the salinity treatment in the early treatment.

The ratio of sucrose to total sugar content in fruits grown under salinity stress increased 1.2–3.6-fold versus that of the control. This increase was observed in all salinity treatments. Interestingly, the increase in sucrose was maintained during the salinity treatments. These results suggest that the increase in sugar content with salinity treatment may be not only a result of concentration, but also due to changes in sugar metabolism in the fruit and/or sugar translocation into the fruit. However, salinity treatment also induced a reduced rate of photosynthesis that affected carbohydrate assimilation (Romero-Aranda et al., 2001). The effect of salinity on the movement of photosynthates from leaves to fruits should be examined.

Salinity stress in the early growth stage of fruits induced more BER than that in the later growth stage. BER is considered to be induced by decreasing Ca^{2+} uptake by the roots and Ca^{2+} transport via the xylem to the fruits (Belda and Ho, 1993; Belda et al., 1996; Ho et al., 1993). BER is caused by a calcium deficiency occurring at the distal end of tomato fruits during the initial stage of fruit development, i.e., within a few weeks after anthesis (Bangerth, 1979; El-Gizawy et al., 1986; Sonneveld and Voogt, 1991; van Goor, 1968; Ward, 1973). Therefore, BER may occur more frequently if the salinity stress is applied during the early stage of fruit development.

The incidence of fruit cracking was reduced by salinity treatment, particularly in the total and late treatments. Fruit cracking is generally associated with the rapid net influx of water and solutes into the fruit when cuticle elasticity and strength are reduced (Peet, 1992). Salinity treatment could inhibit the incidence of fruit cracking by reducing water influx into fruits. In the early treatment, the incidence of fruit cracking was higher than in both the whole and late treatments because of the rapid water influx into fruits following the end of salinity treatment.

Fruit yield per plant, the sugar content of fruit and average fruit fresh weight decreased with increasing planting density, but there was an increase in yield per unit area (with increasing planting density) because of the increased number of stems per unit area (Green, 1980; Saglam and Yazgan, 1995). We evaluated the interaction of salinity and planting density in the culture system of low node-order pinching. In the case of medium- and high-density plantings, the soluble sugar content of the fruit decreased in the whole treatment and the fresh fruit weight decreased in the control, relative to the low-density planting treatment (Fig. 3A).

However, the sugar content and titratable acidity were not affected by the high planting density with the salinity treatments (Table 3). These results agree with those of Heuvelink (1995), Osvald et al. (2001), and Warner et al. (2002). The fruit fresh weight was also unaffected by planting density with salinity treatment, and the soluble sugar content of fruits was maintained above 8.0% (Fig. 3B). Mutual shading as a negative effect of high planting density may have declined because leaf area was reduced by salinity stress (Mulholland et al., 2002).

This study showed that salinity stress induced the inhibition of fruit enlargement and the concentration of sugars. The effects of salinity stress on fruit size and quality were greater when the stress was maintained for the entire or latter stage of fruit development. In addition, we demonstrated that under saline conditions, the effect of planting density on fruit size and quality was smaller than that under non-saline conditions. These results are important for increasing yields without loss of quality in the low node-order pinching and dense planting culture system.

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塩ストレス処理期間および栽植密度が養液栽培トマトの果実の大きさならびに糖含量に及ぼす影響

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NFT を用いた養液栽培トマトにおいて，異なる塩ストレスの処理開始時期，処理期間および栽植密度が，養液栽培トマトの果実収量ならびに果実品質に及ぼす影響について調査した．塩ストレス処理は，培養液に NaCl を添加し，EC を 8.0 $dS \cdot m^{-1}$ に調節することによって行った．NaCl を添加しない対照区は，EC 2.5 $dS \cdot m^{-1}$ とした．平均果実重量は，塩ストレス処理を行わなかった場合と比較して，塩ストレス処理を開花後の果実生育の全期間に行うと約 49%，前半のみの処理で約 73%，後半のみの処理で約 63%となった．可溶性固形物含量（Brix%）は，対照区で 6.1，全期間処理で 9.7，前期処理で 7.9，後期処理で 8.6 となった．尻腐れ果発生率は，全期間処理と前期処理で 30%以上であったのに対して，対照区では 0%，後期処理では 16%であった．果実生育期の中後半から塩ストレス処理を開始することで，対照区より品質が向上し，全期間処理より収量が増加し，尻腐れ果発生率も低くなった．また，塩ストレス処理下では，低栽植密度区（1 m^2 当たり 6.7 個体）と比較して中栽植密度区（1 m^2 当たり 8.3 個体），高栽植密度区（1 m^2 当たり 9.5 個体）において大きな品質低下を伴わずに，単位面積あたりの収量が増加した．