Effects of Uneven Vertical Distribution of Soil Salinity on Blossom-end Rot of Tomato Fruit

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Abstract. Soil salinity influences plant growth and crop yield significantly. Former studies indicated that uneven salt distribution in the root zone could relieve salt stress. But, how uneven salt distribution influences Na^+ and Ca^{2+} concentration in the stem, leaf, and fruit and whether this influence would bring effects on fruit blossom-end rot (BER) still needs to be further studied. Under consideration of this, pot experiment with four treatments, $T_{1:1}$, $T_{1:5}$, $T_{2:4}$, and $T_{3:3}$, was conducted by setting the upper soil layer salinity at 1‰, 1‰, 2‰, and 3‰ and the lower soil layer at salinities of 1‰, 5‰, 4‰, and 3‰, respectively. Compared with the uniform salt concentration in the root zone ($T_{3:3}$ treatment), the incidence of BER in the $T_{1:5}$ and $T_{2:4}$ treatments decreased by 60% and 35%, respectively. The fruit Na^+ concentration and $\text{Na}^+/\text{Ca}^{2+}$ ratio were positively correlated with the incidence of BER. The value of the upper-root selective absorption Ca^{2+} over Na^+ ($S_{\text{Ca}/\text{Na}(\text{upper root})}$) for $T_{1:5}$ was 0.8 times more than that of $T_{1:1}$. The results showed that the incidence of BER was positively correlated with root dry matter and $S_{\text{Ca}/\text{Na}(\text{root})}$ weighted mean salinity. The overall results suggested that uneven salt distribution in the root zone could promote the Ca^{2+} absorption, $\text{Ca}^{2+}/\text{Na}^+$ ratio, and selective absorption Ca^{2+} over Na^+ and consequently decrease the incidence of BER in tomato fruit.

Salt stress adversely affects plant growth and causes 7% decrease in crop yield all over the world (Latef and Chaoxing, 2011; Maggio et al., 2004). Plants suffer from salt stress in the following ways: 1) the decrease in the osmotic potential in the rooting medium makes it difficult for roots to absorb water, 2) the toxic effect of a high concentration of Na^+ triggers a series of toxicity events and causes damage to the systems that absorb and use Ca^{2+} ion, and it negatively affects crop growth and development, because Ca^{2+} is essential for various physi-

ological and biochemical processes (Ebrahimi and Bhatla, 2012; Gama et al., 2007; Hilge, 2012; Munns and Tester, 2008). Furthermore, a high rate of BER occurrence has been associated with a low Ca^{2+} concentration in the fruit tissue (Ho and White, 2005).

Overirrigation with fresh or brackish water before planting is a common practice for growing crops in areas with high salinity (Dong et al., 2010). Using this approach, excess irrigation reduces the surface soil salinity to maintain plant growth. However, this method does not always work because salts will move upward by capillary rise in response to higher potential evapotranspiration and accumulate near the soil surface (Imada et al., 2015; Rengasamy, 2006; Salama et al., 1999). To solve this problem, a capillary barrier (CB) is often chosen to prevent salt accumulation in the surface soil layer, which is a phenomenon of hydraulic for disrupted capillary water continuity (Ittyel et al., 2014). Rooney et al. (1998) found a decrease of 66% in capillary

water moving upward, which led to a lesser soil salt content in the surface by using a CB above the shallow groundwater table. When compared with the treatment without a CB, Ittyel et al. (2012) found that the buried isolation layer could lead to a 24% increase in yield of pepper plants. In addition, recent studies indicated that root of plants could easily penetrate through the isolation layer if it was designed properly (Chen et al., 2016; Dara et al., 2015). Under CB conditions, soil salt always moves downward with water (Zhao et al., 2014, 2016). Once the root penetrates through the layer, the distinct salt concentrations for the upper and lower soil layers could result in the vertical heterogeneous distribution of salinity in the root environment (Qiao et al., 2006; Zhang et al., 2010). The results of former researchers had indicated that when compared with uniform salinity, an unequal salt distribution in the soil would improve the growth of oranges (Zekri and Parsons, 1990), cucumbers (Sonneveld and de Kreij, 1999), tomatoes (Tabatabaie et al., 2004), and *Atriplex nummularia* (Bazihizina et al., 2009). Our former study also showed that an uneven salt distribution in the soil could enhance the growth of tomatoes (Chen et al., 2016). Recent studies of Dong et al. (2008, 2010) showed that under plastic mulching, furrow seeding could increase the cotton yield in saline environments because this method resulted in an unequal salt distribution in the root zone. Previous studies mainly focused on the responses of plants to the horizontal uneven salt distribution in the root zone (Bazihizina et al., 2009; Dong et al., 2008; Kong et al., 2011), whereas there have been few studies on the vertical uneven salt distribution (Bazihizina et al., 2012b; Bingham and Garber, 1970), which was presumably because of difficulties in applying and maintaining the treatments.

The cultivated areas for tomatoes (*Solanum lycopersicum* L.) have increased significantly across the globe, and greenhouse cultivation has become economically important (He et al., 2007). Tomato is one of the most commonly cultivated crops in saline areas, and it is moderately tolerant of salinity ($1.3 \text{ dS}\cdot\text{m}^{-1} < \text{EC}$ of the saturated soil extract $< 6.0 \text{ dS}\cdot\text{m}^{-1}$) (Amjad et al., 2014; Cuartero and Fernández-Munoz, 1998; Lu et al., 2010). Several studies have focused on the effects of salt stress on the growth and yield of tomato plants. Above an EC of $2.5 \text{ dS}\cdot\text{m}^{-1}$, a 10% yield decrease per additional $\text{dS}\cdot\text{m}^{-1}$ was obtained (Bazihizina et al., 2012a). As one of the most important physiological disorders of tomato fruit, BER may reduce the marketable yield by up to 50% and influence the farmer's income (Taylor et al., 2004). Researchers have found that high salinity would increase the incidence of BER (Abdal and Suleiman, 2004; Reina-Sanchez et al., 2005), which was generally believed to result from plasma membrane breakdown in response to a low calcium

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concentration in the fruit tissue (Ho and White, 2005). Soil salinity will make negative effects on the fruit Ca^{2+} absorption and concentration, whereas Ca^{2+} is essential for proper membrane function (Abdal and Suleiman, 2004; de Freitas et al., 2011; Suzuki et al., 2003). It has also been shown that the Ca^{2+} accumulation in fruit was significantly connected to the amount of absorbed water and the transpiration of plants (Keiser and Mullen, 1993; Sun et al., 2013). On the other hand, Na^+ ion toxicity would damage the Ca^{2+} absorption and utilization systems. Bazihizina et al. (2009) and Flores et al. (2002) found that when plant roots were subjected to an unequal salt distribution, the water absorption of the whole plant was higher than when the roots were subjected to uniform salinity and a compensatory increase in water uptake through the untreated part of the plant was observed. In addition, plant sodium content was usually lower in non-uniform treatments when compared with a uniform soil salt distribution (Bazihizina et al., 2009; Dong et al., 2010). We assumed that the above effects, which were brought on by nonuniform salinity, would promote plant Ca^{2+} absorption and concentration. To test our hypotheses, the Ca^{2+} concentration and $\text{Ca}^{2+}/\text{Na}^+$ absorption of different plant organs were analyzed in this study. Some previous studies focused on the influence of Na^+ on absorption and the distribution of Ca^{2+} in the plant organs (Girija et al., 2002). Our former study also discussed the Na^+ and Ca^{2+} distribution in the leaves and fruit (Chen et al., 2016). However, when soil salinity was unevenly distributed in a vertical direction, the Na^+ and Ca^{2+} concentrations in different root parts or the movement of Na^+ and Ca^{2+} in different plant organs has seldom been reported. According to Pitman (1982) and Zheng et al. (2010), the selective uptake of Ca^{2+} over Na^+ ($S_{\text{Ca}/\text{Na}}$) is a useful indicator for salt stress. To test the possibility in the current study, $S_{\text{Ca}/\text{Na}}$ values of different organs under different salinity treatments were investigated.

Previous studies indicated that the occurrence of tomato fruit BER was tightly linked with Ca^{2+} concentration in the fruit tissue (Ho and White, 2005). In addition, more water would be consumed when the plant root zone was under nonuniform salt stress (Bazihizina et al., 2009), and the plant Ca^{2+} concentration was correlated with the water consumed by plants (Keiser and Mullen, 1993). Thus, we speculated that unequal salt distribution in the soil might influence the amount of absorbed Ca^{2+} in plants and could decrease BER rates of tomato fruits. However, the relationship between fruit BER and soil salt distribution as well as the amount of Ca^{2+} absorption by plant root has rarely been studied until now. Previous studies have mostly focused on the relationship between plant growth and heterogeneous salinity in the horizontal direction in the root zone and suggested

that the growth of plants might be related to 1) the lowest saline part of the root zone (Bazihizina et al., 2012b), 2) the highest saline part of the root zone (Lycoskoufis et al., 2005), 3) the mean salinity of root zone (Shalhevet, 1994), and 4) the root dry matter weighted mean salinity of the root zone (Minhas and Gupta, 1993). In this study, the correlation between fruit BER and the upper layer salinity, the lower layer salinity, the mean salinity, and the root dry matter weighted mean salinity (RWMS) were investigated. In addition, assuming that selective absorption of Ca^{2+} over Na^+ ($S_{\text{Ca}/\text{Na}}$) might affect the ion concentration and the incidence of fruit BER in tomatoes, the values of root dry matter and selective uptake of Ca^{2+} over Na^+ weighted mean salinity (RSWMS) were also be taken into consideration when the relationship between fruit BER and the soil salinity were analyzed.

Based on the theoretical understanding of plant responding to uneven salt stress in the root -zone, we hypothesized that 1) an unequal salt distribution in the vertical direction could promote plant Ca^{2+} absorption, 2) the selective absorption of Ca^{2+} over Na^+ ($S_{\text{Ca}/\text{Na}}$) could affect the ion concentration and the incidence of BER in tomato fruit.

Materials and Methods

Growing conditions

This experiment was conducted from April to June 2014 in a greenhouse located at the Key Laboratory of Efficient Irrigation-Drainage and Agricultural Soil-Water Environment in Nanjing, Jiangsu Province, Southern China. The polyethylene pots having a height of 42 cm, upper and lower diameters of 30 and 25 cm, respectively, were used for the experiment. A type of sandy soil with two different salt contents (0.94‰ and 6.22‰, respectively) was collected from the coastal area of Dongtai, Jiangsu Province. For the salt distribution in the upper and lower layers, four treatments, i.e., $T_{1:1}$, $T_{1:5}$, $T_{2:4}$, and $T_{3:3}$, which represented the soil salt content of the upper soil layer: lower soil layer at the initiation of treatment were 1‰:1‰, 1‰:5‰, 2‰:4‰ and 3‰:3‰, respectively, were used in this study. Peat substrate (GB-Pindstrup Substrate No.1, pH 6.0) was mixed with the original soil at a rate of 32.5 g per pot to obtain new mixed soil with a bulk density of 0.94 g·cm⁻³. In addition, 0.326 g of urea ($\text{CO}(\text{NH}_2)_2$), 2.5 g of organic fertilizer (4% N, 4% P, and 4% K), 0.13 g of potassium sulfate (K_2SO_4), and 0.38 g of potassium dihydrogen phosphate (KH_2PO_4) per pot were mixed with the soil to meet nutrient requirements during the plant growing period. The soil in the pot was divided by a straw layer into upper and lower soil layers with weights of 8.6 and 12.9 kg, respectively. It is noteworthy that the total soil salinity was $T_{1:5} > T_{2:4} > T_{3:3}$, as we know that uneven salt distribution could relieve the salt stress in the root zone;

thus, the soil salinity increase from $T_{3:3}$ to $T_{1:5}$ will bring a negative effect on the uneven salt distribution method. That is to say, if $T_{1:5}$'s plant growth was affected less by salt stress than $T_{3:3}$, it means that uneven salt distribution is more effective because of the higher total soil salinity. In this research, we will ignore the difference in total soil salinity between $T_{1:5}$, $T_{2:4}$, and $T_{3:3}$, and focus on the situation of salt distribution in the soil. The weight, thickness, and depth of the straw layer were ≈ 50 g, 3 cm and 17 cm, respectively. The water-holding capacity (θ_f) of the mixed soil was 37% (Vol.). The pots were spaced at 50 × 50 cm². Each treatment contained 12 replications, and four plants were selected randomly when the harvesting experiment was carried out. Tomato (*Solanum lycopersicon* L. var. Yazhoufenwang) seeds were sown on Mar. 5 when the third true leaf of the seedlings expanded (20 Apr.); seedlings of similar height were selected for transplanting to the pots. Tomato plants were topped by pitching the terminal bud of the main stem when the third flower cluster was visible. Pest and weed controls were conducted according to local commercial practices.

Sampling and analyses

Water consumption. 5TE probes (Decagon Devices Inc., Pullman, WA) were connected to an EM50 data logger (Decagon Devices Inc.) and buried at the center of the upper and lower soil layers, which were chosen for continuously monitoring volumetric soil water content (θ_v). Water consumption was calculated from θ_v , and the volume of the soil. θ_v was kept around 50% of the water-holding capacity (θ_f) from transplanting to 26 d after transplanting (DAT). From 27 DAT to the end of the experiment, the lower and upper limit of θ_v in each pot was set to 60% of θ_f and 80% of θ_f , respectively.

Fruit BER. Tomato plants were harvested on June 15. The number and fresh weight of normal or BER fruit were measured from four samples of each treatment, respectively. Incidence of BER and BER fruit fresh yield (BER FY) percentage were calculated as:

$$\text{Incidence of BER} = \frac{n_1}{n_1 + n_2} \quad [1]$$

$$\text{BER FY percentage} = \frac{y_1}{y_1 + y_2} \quad [2]$$

where, n_1 and y_1 represents the number and fresh weight of BER fruit, respectively, and n_2 and y_2 represents the number and the fresh weight of non-BER fruit, respectively.

Dry biomass of plant organs. After washing carefully with tap water, root systems in upper and lower soil layers were collected, and the aboveground part was also separated into stems, leaves, and fruits. Dry biomass of plant samples was determined after oven drying the samples at 70 °C until they reached a constant weight.

Na^+ and Ca^{2+} concentrations in organs and soil. After drying the samples, organs

Table 1. Root dry matter and water consumption in the upper soil layer, lower soil layer, and whole soil layer for tomato plants subjected to T_{1:1}, T_{1:5}, T_{2:4}, and T_{3:3} treatments.

Treatment	Upper soil layer		Lower soil layer		Whole soil layer	
	Root dry matter (g)	Water consumption (L)	Root dry matter (g)	Water consumption (L)	Root dry matter (g)	Water consumption (L)
T _{1:1}	5.78 ± 0.94 ab	18.58 ± 0.38 b	4.23 ± 0.95 a	15.39 ± 0.52 a	10.01 ± 1.73 a	33.97 ± 0.22 a
T _{1:5}	6.93 ± 1.30 a	25.18 ± 0.33 a	1.65 ± 0.41 b	8.44 ± 0.12 c	8.57 ± 1.49 ab	33.62 ± 0.23 a
T _{2:4}	7.44 ± 1.20 a	16.11 ± 0.18 c	2.64 ± 0.55 b	7.86 ± 0.24 d	10.08 ± 1.73 a	23.97 ± 0.34 b
T _{3:3}	4.82 ± 1.26 b	12.38 ± 0.47 d	1.71 ± 0.65 b	10.57 ± 0.34 b	6.53 ± 1.71 b	22.94 ± 0.66 c

The treatment symbols T_{1:1}, T_{1:5}, T_{2:4}, and T_{3:3} represent the soil salt content of the upper and lower layers at the initiation of the treatment, which were 1‰ and 1‰, 1‰ and 5‰, 2‰ and 4‰, and 3‰ and 3‰, respectively. The data are mean ± SD (n = 4). Values followed by different superscript letters within a row are significantly different at 5% according to Duncan's multiple range tests, whereas the lowercase letters indicate P < 0.05, and uppercase letters indicate a P < 0.01.

Fig. 1. (A) Number of blossom-end rot (BER) fruit per plant, (B) number of non-BER fruit per plant, (C) incidence of BER, (D) BER fruit fresh yield (BER FY) per plant, (E) non-BER FY, and (F) BER FY percentage under different treatments. The treatment symbols T_{1:1}, T_{1:5}, T_{2:4}, and T_{3:3} represent the soil salt contents of the upper and lower layers at the initiation of the treatment, which were 1‰ and 1‰, 1‰ and 5‰, 2‰ and 4‰, and 3‰ and 3‰, respectively. Each value is the Mean ± SD (n = 4). The different letters on the tops of column for the same soil layer indicate significant differences between treatments at 5% (*) or (**) according to Duncan's multiple range test.

were grinded to pass through a 2-mm sieve. After 1 g of dried samples was digested with nitric acid and perchloric acid, the solution was diluted with deionized water to 100 mL. Soil samples were taken from the gap of the root, after

air-drying the samples, soil was grinded to pass through a 1-mm sieve, and 2 g of soil were mixed with 10 mL of demonized water. Na⁺ and Ca²⁺ concentrations in organs and soil were measured using inductively coupled plasma optical emission

spectrometry (ICP-OES, 710 Series; Agilent Technologies).

Selective uptake of Ca²⁺ over Na⁺. According to Pitman (1982), the selective uptake of Ca²⁺ over Na⁺ in the upper roots, lower roots, stem, fruits, and leaves was calculated as

$$S_{Ca/Na(upper-root)} = \frac{Ca_{upper-root}/Na_{upper-root}}{Ca_{upper-soil}/Na_{upper-soil}} \quad [3]$$

$$S_{Ca/Na(lower-root)} = \frac{Ca_{lower-root}/Na_{lower-root}}{Ca_{lower-soil}/Na_{lower-soil}} \quad [4]$$

$$S_{Ca/Na(stem)} = \frac{Ca_{stem}/Na_{stem}}{Ca_{upper-root}/Na_{upper-root}} \quad [5]$$

$$S_{Ca/Na(fruit)} = \frac{Ca_{fruit}/Na_{fruit}}{Ca_{stem}/Na_{stem}} \quad [6]$$

$$S_{Ca/Na(leaf)} = \frac{Ca_{leaf}/Na_{leaf}}{Ca_{stem}/Na_{stem}} \quad [7]$$

where $Ca_{upper-soil}$, $Ca_{lower-soil}$, $Ca_{upper-root}$, $Ca_{lower-root}$, Ca_{stem} , Ca_{fruit} , and Ca_{leaf} represent the Ca^{2+} concentrations of the upper layer soil, lower soil layer, upper roots, lower roots, stems, fruits, and leaves, respectively; $Na_{upper-soil}$, $Na_{lower-soil}$, $Na_{upper-root}$, $Na_{lower-root}$, Na_{stem} , Na_{fruit} , and Na_{leaf} represents the Na^+ concentrations of the upper layer soil, lower soil layer, upper roots, lower roots, stems, fruits, and leaves, respectively.

Different formats to represent heterogeneous salinity in the root zone. Surface salinity and the highest salinity (HS) were the upper and lower layer soil salt content, respectively. Arithmetic mean salinity (AMS) was calculated as the average of the upper and lower soil salt content. Refer to Minhas and Gupta (1993), RWMS was calculated as:

$$RWMS = \frac{r_1 \times s_1 + r_2 \times s_2}{r_1 + r_2} \quad [8]$$

RSWMS

$$RSWMS = \frac{r_1 \times S_{Ca/Na}^1 \times s_1 + r_2 \times S_{Ca/Na}^2 \times s_2}{r_1 \times S_{Ca/Na}^1 + r_2 \times S_{Ca/Na}^2} \quad [9]$$

where r_1 and r_2 are the dry weights of the upper and lower roots, respectively, $S_{Ca/Na}^1$, $S_{Ca/Na}^2$ is the selective uptake of Ca^{2+} over Na^+ from the soil in upper and lower roots, respectively, and s_1 and s_2 are the salt contents of the upper and lower soil layers, respectively.

Statistical analysis

One-way analysis of variance was performed using the general linear model-univariate procedure with SPSS 13.0 software (SPSS, Chicago, IL). The mean values of each treatment were compared using Duncan's multiple range test when significant differences were detected. When appropriate, the Student's *t* test was applied to compare treatment means. The significant level was $P < 0.05$. Linear regression was also carried out in SPSS.

Results

As shown in Table 1, when compared with $T_{3:3}$ (4.82 g), the upper-root dry matter

of $T_{1:5}$ (6.93 g) and $T_{2:4}$ (7.44 g) was significantly higher. For the root dry matter of the lower layer, no difference was found between $T_{1:5}$, $T_{2:4}$, and $T_{3:3}$, whereas all of them were significantly lower compared with $T_{1:1}$. Water consumption of the upper soil layer significantly decreased from 25.18 L ($T_{1:5}$) to 18.58 L ($T_{1:1}$), 16.11 L ($T_{2:4}$), and 12.38 L ($T_{3:3}$), respectively. The water consumption of the lower layer in $T_{1:1}$ was 15.39 L, which was significantly higher compared with the other three treatments. When considering the whole root zone, $T_{1:1}$ and $T_{1:5}$ consumed significantly more water compared with $T_{2:4}$ and $T_{3:3}$.

BER fruit number (A), non-BER fruit number (B), incidence of BER (C), BER fresh yield (D), non-BER fresh yield (E), and BER FY percentage (F) are shown in Fig. 1. When soil salt was equally distributed, with an increase in the average salt concentration in the whole soil profile, the average number and fresh yield of BER fruit dramatically increased from 0.25 and 8.42 g of $T_{1:1}$ to 4.00 and 145.40 g of $T_{3:3}$. Similarly, the incidence of BER and BER FY percentage significantly increased from 1.79% and 1.70% of $T_{1:1}$ to 35.24% and 39.12% of $T_{3:3}$, respectively. As shown in Fig. 1, the incidence of BER and BER FY percentage of

$T_{1:5}$ was 14.24% and 20.91%, respectively, and those percentages were significantly lower as compared with $T_{3:3}$.

Na^+ and Ca^{2+} concentrations in different plant organs are shown in Fig. 2. The increase in salinity had a significant effect on plant Na^+ concentration. When soil salt increased from 1‰ of $T_{1:1}$ to 3‰ of $T_{3:3}$ on an average, the Na^+ concentrations in the stems, leaves, and fruits significantly increased from 0.108, 0.234, and 0.058 mM to 0.540, 1.308, and 0.168 mM, respectively. When the average salt content in the whole soil layer was similar, the Na^+ concentration in the above-ground parts decreased as the soil salt distribution diversity increased. The Na^+ concentrations in stems, leaves, and fruits for $T_{1:5}$ were 39%, 65%, and 69% of $T_{2:4}$, and 31%, 54%, and 58% of $T_{3:3}$, respectively, and the differences were significant. In addition, the unequal salt distribution had a significant effect on fruit Ca^{2+} concentration with a 47.6% higher in $T_{1:5}$ than $T_{3:3}$. The upper root Ca^{2+} concentration of $T_{1:1}$ and $T_{1:5}$ was higher compared with $T_{2:4}$ and $T_{3:3}$, whereas the lower roots of $T_{1:5}$ showed the lowest amounts among the treatments.

The correlation between Ca^{2+} and Na^+ concentrations and incidence of BER were analyzed when the data from four different treatments were pooled in this experiment

Fig. 2. (A) Na^+ and (B) Ca^{2+} concentration in plant leaves, stems, fruits, upper-part roots, and lower-part roots of $T_{1:1}$, $T_{1:5}$, $T_{2:4}$, and $T_{3:3}$. The treatment symbols $T_{1:1}$, $T_{1:5}$, $T_{2:4}$, and $T_{3:3}$ represent the soil salt contents of the upper and lower layers at the initiation of the treatment, which were 1‰ and 1‰, 1‰ and 5‰, 2‰ and 4‰, and 3‰ and 3‰, respectively. Each value is the Mean $\pm$ SD ($n = 4$). The different letters on the tops of the column for the same soil layer indicate significant differences in the same organ between treatments at 5% (*) or 1% (**) according to Duncan's multiple range test.

(Fig. 3). A significant negative relationship was found between the Ca^{2+} concentration and the incidence of BER ($P < 0.05$). In addition, positive linear relationships between the incidence of BER and Na^+ concen-

Fig. 3. The correlation between incidence of blossom-end rot (BER) and fruit Ca^{2+} concentration, Na^+ concentration, and $\text{Na}^+/\text{Ca}^{2+}$ ratio. The analysis was based on 16 plants that belonged to $T_{1:1}$, $T_{1:5}$, $T_{2:4}$, and $T_{3:3}$. The treatment symbols $T_{1:1}$, $T_{1:5}$, $T_{2:4}$, and $T_{3:3}$ represent the soil salt contents of the upper and lower layers at the initiation of the treatment, which were 1‰ and 1‰, 1‰ and 5‰, 2‰ and 4‰, and 3‰ and 3‰, respectively. Equation and correlation coefficients of the three relationships are shown in this figure. *Significance at $P < 0.05$ and ** significance at $P < 0.01$.

tration and the $\text{Na}^+/\text{Ca}^{2+}$ ratio were also found ($P < 0.01$).

As shown in Table 2, the $\text{Ca}^{2+}/\text{Na}^+$ of $T_{1:5}$ in the upper root was the highest among the treatments, whereas the lower root parts showed the opposite trend. The $\text{Ca}^{2+}/\text{Na}^+$ of the aboveground biomass decreased with the increase in average soil salinity, where the $\text{Ca}^{2+}/\text{Na}^+$ of stems, leaves, and fruits of $T_{1:1}$ showed the highest values among the treatments. When the average salt content in the soil profile was similar, unequal salt distribution for treatment $T_{1:5}$ led to a significantly higher $\text{Ca}^{2+}/\text{Na}^+$ in stems, leaves, and fruits when compared with $T_{2:4}$ and $T_{3:3}$.

As shown in Table 3, selective uptake of Ca^{2+} over Na^+ ($S_{\text{Ca}/\text{Na}}$) in the upper roots of $T_{1:5}$ was 82.7%, 72.0%, and 166.1% more than $T_{1:1}$, $T_{2:4}$, and $T_{3:3}$, respectively, which suggested that the upper-root system of $T_{1:5}$ suffered less salt stress compared with the other treatments. The opposite trend was observed in the lower-root system and $S_{\text{Ca}/\text{Na}}(\text{lower root})$ of $T_{1:5}$ significantly decreased to 42.76, whereas the values for $T_{1:1}$, $T_{2:4}$, and $T_{3:3}$ were 85.56, 62.70, and 91.52, respectively.

As shown in Fig. 4, the correlation between incidence of BER and SS, RWMS, RSWMS was significant, and the highest correlation coefficient was found in RSWMS ($R^2 = 0.91$). In addition, there was no significant relationship between the incidence of BER and AMS or the HS.

Discussion

Increased salinity will promote the occurrence of fruit BER (Van Ieperen, 1996). Reina-Sanchez et al. (2005) found the average number of BER fruits in four tomato cultivars increased with salinity changing from 2‰ in 0 mM Na^+ conditions to 16‰ in 75 mM. In our study, under a homogeneous distribution of salt in the vertical direction of the root zone, the incidence of BER and the BER FY percentage of $T_{3:3}$ (soil salinity was 3‰) were 35.3% and 39.1%, respectively, and those values were significantly higher than that of $T_{1:1}$ (soil salinity was 1‰, Fig. 1).

While under a similar salt concentration throughout the entire soil profile and with an increased salt concentration in the upper soil profile, the incidence of BER for $T_{3:3}$ was 147% and 53% higher as compared with $T_{1:5}$ and $T_{2:4}$. It is generally believed that fruit BER is linked to a deficiency of Ca^{2+} concentration in fruit tissue (Ho and White, 2005), especially in the distal end of the fruit (Ho et al., 1995). In the current study, when average soil salinity increased from 1‰ ($T_{1:1}$) to 3‰ ($T_{1:5}$, $T_{2:4}$ and $T_{3:3}$), the fruit Ca^{2+} concentration significantly decreased from 0.140 mM to 0.086, 0.062, and 0.058 mM, respectively (Fig. 2). In addition, an uneven salt distribution in the vertical direction had a significant effect on fruit Ca^{2+} concentration with a 40.6% increase in $T_{1:5}$ compared with $T_{3:3}$ (Fig. 1). Fruit Ca^{2+} in different treatments showed the opposite trend compared with the incidence of BER, and significant negative linear relationships between them were observed ($R^2 = 0.77$, Fig. 3). Morard et al. (2000) proposed that Ca^{2+} uptake and movement in the plant was tightly linked with water consumption and transpiration. The increase in salinity had a significant effect on water consumption in this study. Under uniform distribution of salt in the root zone, $T_{1:1}$ consumed 48.1% more water when compared with $T_{3:3}$ (Table 1). There was a similar concentration in the whole root profile, and tomato water consumption was also significantly affected by a vertical distribution of salt in the root zone. As shown in Table 1, water consumption decreased with the increasing salt concentration in the upper soil profile ($T_{2:4}$ and $T_{3:3}$ compared with $T_{1:5}$). When soil salinity was distributed unevenly in the vertical direction, seldom former researchers focused on the water consumption in different root part, but when soil salinity was uneven distributed in the horizontal direction, Bazihizina et al. (2009) and Flores et al. (2002) pointed out that the main reason for the plant to consume more water in the uneven salt distribution environment might be a compensatory increase in water uptake through the salt-free

Table 2. $\text{Ca}^{2+}/\text{Na}^+$ ratio of the upper root layer, lower root layer, stems, leaves, and fruits in $T_{1:1}$, $T_{1:5}$, $T_{2:4}$, and $T_{3:3}$.

Treatment	Upper root $\text{Ca}^{2+}/\text{Na}^+$ ratio	Lower root $\text{Ca}^{2+}/\text{Na}^+$ ratio	Stem $\text{Ca}^{2+}/\text{Na}^+$ ratio	Leaf $\text{Ca}^{2+}/\text{Na}^+$ ratio	Fruit $\text{Ca}^{2+}/\text{Na}^+$ ratio
$T_{1:1}$	4.89 ± 1.60 b	5.35 ± 1.35 ab	6.22 ± 0.81 a	12.33 ± 0.77 a	4.14 ± 0.48 a
$T_{1:5}$	8.93 ± 4.05 a	2.67 ± 0.64 b	3.22 ± 0.23 b	5.90 ± 0.81 b	1.53 ± 0.29 b
$T_{2:4}$	5.19 ± 1.91 b	3.92 ± 1.15 bc	2.25 ± 0.42 c	0.52 ± 0.17 c	0.77 ± 0.13 c
$T_{3:3}$	3.36 ± 0.62 b	5.72 ± 0.96 a	1.47 ± 0.02 d	0.26 ± 0.01 c	0.63 ± 0.20 c

The treatment symbols $T_{1:1}$, $T_{1:5}$, $T_{2:4}$, and $T_{3:3}$ represent the soil salt contents of the upper and lower layers at the initiation of the treatment, which were 1‰ and 1‰, 1‰ and 5‰, 2‰ and 4‰, and 3‰ and 3‰, respectively. Data are the mean ± SD ($n = 4$). Values followed by different superscript letters within a row are significantly different at 5% according to Duncan's multiple range tests, whereas the lowercase letters mean $P < 0.05$ and uppercase letters mean $P < 0.01$.

Table 3. Upper and lower root, stem, leaf, and fruit $\text{Ca}^{2+}/\text{Na}^+$ selectively absorption.

Treatment	$S_{\text{Ca}/\text{Na}}(\text{upper-root})$	$S_{\text{Ca}/\text{Na}}(\text{lower root})$	$S_{\text{Ca}/\text{Na}}(\text{stem})$	$S_{\text{Ca}/\text{Na}}(\text{leaf})$	$S_{\text{Ca}/\text{Na}}(\text{fruit})$
$T_{1:1}$	78.23 ± 25.52 b	85.56 ± 21.56 ab	1.42 ± 0.64 a	2.00 ± 0.18 a	0.68 ± 0.11 a
$T_{1:5}$	142.99 ± 64.86 a	42.76 ± 10.23 c	0.43 ± 0.21 b	1.83 ± 0.21 a	0.48 ± 0.11 b
$T_{2:4}$	83.11 ± 30.60 b	62.70 ± 18.36 bc	0.47 ± 0.17 b	0.25 ± 0.10 b	0.35 ± 0.09 b
$T_{3:3}$	53.73 ± 9.97 b	91.52 ± 15.35 a	0.45 ± 0.07 b	0.18 ± 0.01 b	0.43 ± 0.14 b

The treatment symbols $T_{1:1}$, $T_{1:5}$, $T_{2:4}$, and $T_{3:3}$ represent the soil salt contents of the upper and lower layers at the initiation of the treatment, which were 1‰ and 1‰, 1‰ and 5‰, 2‰ and 4‰, and 3‰ and 3‰, respectively. Data are the mean ± SD ($n = 4$). Values followed by different superscript letters within a row are significantly different at 5% according to Duncan's multiple range tests, whereas lowercase letters mean $P < 0.05$ and upper-case letters mean $P < 0.01$.

Fig. 4. The relationship between the incidence of blossom-end rot (BER) with the upper layer salinity (US), root dry matter weighted mean salinity (RWMS), root dry matter and $S_{Ca/Na}$ weighted mean salinity (RSWMS) as well as the formulas between them, from A to C, are shown. The incidence of BER for different treatments tested an average number of four plants that belonged to the same treatment. **Significance of lines at $P < 0.01$.

part when the other root system experienced to salinity. Thus, we believe that there may be similar compensatory mechanism for water absorption in horizontal and vertical non-uniform salinity. The water consumption in the upper layer of T_{1:5} was 103.3% higher than the upper layer of T_{3:3} (Table 1).

It is noteworthy that in this research a better indicator for the incidence of BER was Na^+/Ca^{2+} in the fruit ($R^2 = 0.88$, $P < 0.01$), and it proved to be more effective than Ca^{2+} alone ($R^2 = 0.77$, Fig. 3). Negative effects induced by Na^+ on Ca^{2+} uptake systems have long been recognized (Demidchik and Maathuis, 2007; Ebrahimi and Bhatla, 2012; Hilge, 2012). In this study, as shown in Fig. 2, Ca^{2+} decreased with the increase in the Na^+ concentration in the fruit, and there was a significant negative correlation between fruit Na^+ and Ca^{2+} concentration. Under the uniform distribution of salt in the root zone, with the increased salt concentration, the fruit Na^+ concentration increased from 0.058 mM in T_{1:1} to 0.164 mM in T_{3:3}, whereas a 58.0% decrease was found in the fruit Ca^{2+} concentration. Under the similar salt concentration in the whole soil profile, soil salt distribution also had a significant effect on the Na^+ and Ca^{2+} concentration in the fruit when the soil salt content of the upper soil profile increased

from 1‰ (T_{1:5}, nonuniform distribution of salt in the root zone) to 3‰ (T_{3:3}, uniform distribution of salt in the root zone). There was a 65.8% increase in the Na^+ concentration as well as a 32.2% reduction in the Ca^{2+} concentration in tomato fruits.

The selective uptake of Ca^{2+} over Na^+ ($S_{Ca/Na}$) of the upper roots, lower roots, stems, leaves, and fruit is shown in Table 3. $S_{Ca/Na}$ of the upper roots for T_{1:5} were significantly higher compared with T_{3:3}, whereas a significantly lower value of T_{1:5} compared with T_{3:3} were observed in the lower root parts. As shown in Table 3, $S_{Ca/Na}$ decreased with the increase in salt content in the soil, and similar results were found by Pitman (1982) and Zheng et al. (2010). Interestingly, it was observed that the upper root $S_{Ca/Na}$ for T_{1:5} were 1.83 times higher than T_{1:1}, even though both soil layers contained a similar salt concentration. This phenomenon could be partially explained by the higher water consumption in the upper soil layer of T_{1:5} compared with T_{1:1} (Table 1) because Ca^{2+} uptake was tightly linked with water movement (Keiser and Mullen, 1993). On the other hand, we also speculated that the difference between $S_{Ca/Na}$ (upper root) of T_{1:1} and T_{1:5} might be related to some internal mechanism, and this speculation needs to be further investigated in the future study.

A significant correlation was observed between fruit incidence of BER and SS in Fig. 4A ($R^2 = 0.82$, $P < 0.01$), and a similar result was obtained by Bernstein and Francois (1973), who indicated that the surface soil salinity was the key factor driving water consumption by the plants. On the other hand, Minhas and Gupta (1993) held the opinion that plant growth was affected by RWMS of the soil. Similarly, in the current study, it was inferred that the relationship between incidence of BER and RSWMS was also significant ($R^2 = 0.654$, $P < 0.01$). Interestingly, the relationship between the incidence of fruit BER and RSWMS was closer than the other soil salinity indicators (Fig. 4, $R^2 = 0.91$, $P < 0.01$), which indicated that the RSWMS might be a good indicator to represent the salinity stress degree in the root zone. A significant decrease in RSWMS was also concluded when soil salinity changed from even distribution (T_{3:3}) to uneven distribution (T_{1:5}, Fig. 4, $P < 0.01$), which represents that the uneven salt distribution could relieve the salt stress by promote root growth and selective uptake of Ca^{2+} over Na^+ , and then decreases the incidence of tomato fruit BER. Based on the results of this research, we believe that an uneven salt distribution in the vertical direction could prevent tomato fruit from BER, and increase the economic yield and also the farmer's income.

Conclusion

In summary, the present study demonstrated that increased soil salinity in the root zone would lead to high incidence of fruit BER, but if soil salinity was unevenly distributed in the vertical direction (T_{1:5}), the

occurrence of BER decreased. When soil salinity was unevenly distributed in the vertical direction, the upper-root system selectively absorbed more Ca^{2+} instead of Na^+ , which was the key factor underlying the high Ca^{2+}/Na^+ ratio in fruit, and that high ratio consequently resulted in a lower incidence of BER. In addition, the RSWMS might be the best indicator for fruit BER incidence.

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