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Effect of salt stress by "onsen" water on plant growth and fruit quality of tomato cv. Reika in pot soil

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Abstract: Salt stress often can enhance the fruit quality of tomatoes. Seawater is one of the substrates used by growers. However, utilization of seawater on tomato production is difficult in the hinterland as it is far away from the seaside. Some "onsen" water also show a high salt concentration (2%). Therefore, it could also be used as a substrate of salt stress treatment. In this study, salt stress was provided by Yupoka "onsen" water, and the effects of different nutrient ECs on plant growth and fruit quality of tomatoes were investigated. Tomato plants 'Reika' were grown in pot soil, and nutrients with EC 2, 4, 8 and 12 mS/cm were applied at the time of irrigation. The fruits were harvested at turning stage until the 3rd truss. Soil salinity attained EC 3.6, 6.7, 12.8, and 15.6 mS/cm. SSC, organic acid, dry matter and NO₃⁻ increased by 50, 79, 50 and 27%, respectively at EC12 mS/cm while, weight, size, and water content decreased up to 40, 20, and 4%, respectively. However, fruit cracking did not occur apparently. Most of the plant growth parameters were reduced.

1. Introduction

Tomato (*Solanum lycopersicum*) is one of the most widely cultivated horticultural crops in the world (FAO, 2016). Tomato fruits are rich in minerals, vitamins, essential amino acids, sugars and dietary fibres and thus contribute to a healthy, well-balanced diet. The majority of tomato fruits produced are consumed in processed form such as peeled tomato (whole or diced), juices, sauce and ketchup, whose manufacture often requires peel removal (Shankara *et al.*, 2005; Rock *et al.*, 2012). Improvement of fruit quality is an urgent issue for tomato growers and consumers (Ho, 1999; Lu *et al.*, 2019). Sugars and organic acids are the most important factors for determining the fruit quality (Lu *et al.*, 2019). Soluble Solids Content (SSC) of ripe tomato fruit is usually 3 to 5% and can reach values over 10% (Balibrea *et al.*, 2006; Gautier *et al.*, 2010). In Japan, "Shio" or salt tomatoes, are cultivated in the area of Uto and Yatsushiro in Kumamoto Prefecture. They are grown on drained land, rich in

salt and other minerals. The wholesale price of "Shio" tomato (have SSC of 10% or higher) is often ten times higher than common tomatoes (http://higoayuminokai.co.jp/sawamura.html).

High electrical conductivity (EC) treatment is a well-known technique to increase SSC of tomato fruit because the decreased osmotic potential of nutrient solution restricts transport of water to fruit (Adams et al., 1991; Cuartero and Fernandez-Munaoz, 1999; Shabala et al., 2012). However, plant exposed to salt stress for long time suffer from phyto-toxicity due to NaCl accumulation. This leads to nutrition imbalance and the uptake of some ions is inhibited (Zhang et al., 2017). Amjad et al. (2014) showed a linear decline of the absorption of macro and micro ions by fruit as EC and truss position become higher. The plant growth and fruit yield began to decline when the nutrient solution exceeded EC 2.5-4.0 mS/cm (Bustomi et al., 2014). In previous studies, EC 5.5 m/.cm treatment reduced the tomato yield by 22.4~31.1%, 12.6~28.0% and 11.7~27.3% in 2012, 2013 and 2014, respectively (Zhai et al., 2015), but SSC increased (Oztekin and Tuzel, 2011; Zhang et al., 2017). Other studies reported in soil-less tomato culture that SSC increased while fruit weight decreased at high salinity (Magán et al., 2008; Kamrani et al., 2013). Although tomato growers commonly use NaCl or seawater to increase EC, it is well known that some "onsen" like Haguro has about 2% of NaCl. Therefore, it may be used as an alternative source of salt stress. This research was conducted to assess if high quality tomato fruit can be grown under salt stress by "onsen" water.

2. Materials and Methods

Four different salt stress treatment (EC 2, 4, 8, and 12 mS/cm) were evaluated on tomato plant 'Reika' during spring to summer in 2016. "Onsen" water obtained from Yupoka in Haguro town, Yamagata/Japan was used as it has a high salty level (EC 40 mS/cm of EC, 2% of NaCl and pH 7.2). Initially, the seeds were sown on moist papers in petri dishes on 5 April, 2016 and maintained in a controlled growth chamber (24°C; 14/10 h light/dark photoperiod; RH 45-50%) to induce germination. On 08 April, the germinated seeds were transferred in cell tray (100 ml) in a glass-house and twenty days after (on 28th April), the seedlings were transplanted into 500 ml pots filled with a planting medium (Baido 300 g containing 0.22 g/l of nitrogen). The seedlings were

maintained in the same glass-house (18.7°C; RH 76.8%). On 17 May, twenty tomato seedlings with 5-7 expanded leaves were further transplanted into plastic pots (25 l) containing Baido 300 g and 300 g of organic fertilizer with 3% of nitrogen (Pro-Bokash-KantoNosan, Tochigi) and maintained in a greenhouse. One month (On 16th June) after transplanting (flowering stage), the plants were treated with different concentration of "onsen" water (EC 2, 4, 8, and 12 mS/cm) replicated five times. The experiment was laid out in Randomized Complete Block Design (RCBD). The EC 2 was 100 g of Hyponex NPK 6-10-5 diluted in 100 l of water. For other treatments (EC 4, 8 and 12), "onsen" water was added into EC 2. The EC meter (LAQUAtwin EC, Horiba, Tokyo) was used to adjust the solution EC. The applied solution was EC 2±0.1, EC 4±0.1, EC 8±0.1, and EC 12±0.1. The nutrient solutions were prepared every week.

Watering was done daily using tap water during the first month (1.8 l/pot). For control of soil/root insects, G F Orthoran (Bifenthrin) insecticide was applied once during the season at rate of 5 g/pot. While for the control of above ground insects, Frutrifol+Tebuconazole, best guard (1 g/l) mixed with a sticker as adjuvant (0.5 ml/l) was also applied every two weeks. Plants were kept as single stem with five trusses and five fruits per truss. Fruits were harvested from three trusses by taking two fruits on each at green maturity until the end of harvest (4 Aug. 2016). The daily average temperature was 24.1°C and RH was 74.2% in the green-house during the growing season.

Plant height, stem size, leaf size, thickness, and SPAD were measured once a week during the plant cultivation from first "onsen" water application until first harvesting. After "onsen" water application, approximately 2 I of tap water were applied once a week into soil pots, and the exudate was collected. EC, pH, NaCl and NO₃-ion concentrations in the exudate were measured using EC meter (LAQUAtwin EC, Horiba, Tokyo), pH meter (LAQUAtwin pH, Horiba, Tokyo), nitrate ion meter (LAQUAtwin NO,, Horiba, Tokyo) and, NaCl meter (CM-14 P, TOA, Tokyo). For the chemical analysis (SSC, organic acid and NO₂-ion), harvested fruits with same skin color selected using Color Reader (CR-10, Konica Minolta, Tokyo) were cut into half longitudinally. Thereafter, samples were taken transversally using cork borer, grinded in the mortar and filtrated by tissues filter. The extracts were centrifuged at 6000 rpm for one minute. SSC, organic acid and NO3⁻were measured by Digital refract-meter (PR-101, Atago, Tokyo), organic acid meter (PAL-BXIACID F5, Atago, Tokyo) and nitrate ion meter (LAQUAtwin NO₃⁻ B-341, Horiba, Tokyo) respectively.

Before measurement, the respective meters were opened and the glass electrodes calibrated with distilled water/or standard solution followed by rinsing with distilled water and wiping using paper towels. Extracted juice per sample was filled slowly on the glass electrode without bubbling, and the measurements were recorded. Prior to measuring, the juice for organic acid was diluted indeionized water using a ratio of 1:50 (0.1 ml of juice into 4.9 ml of desalted water). SSC was expressed in % Brix, organic acid in %, and NO₃ in ppm.

Half of the harvested fruit samples were frozen at -20°C for ten days. The samples were dried using Eyela Freeze Dryer FDU-540. The wet and dry samples were weighed in order to determine fruit water and dry matter content. The dried ones had been powdered in mortar using liquid nitrogen. One gram of the powdered sample was put in a crucible, and incinerated at 200°C for 2 hours, 550°C for 10 hours, and 200°C for 2 hours, respectively. Fruit ash content was calculated by weighing the sample.

Data analysis were performed using the Statistical Analysis Software package, GenStat 19th edition at 5% level of significance. Least significant difference test (LSD) was used to separate treatment means.

3. Results

The average temperature was 24°C and RH 74% in the green house during tomato cultivation. The difference in plant height among salt stress treatment was observed after about three weeks "onsen" water application. Fruit SSC, organic acid and NO,⁻ increased by about 50, 79 and 26.5%, respectively in EC12 mS/cm while SSC: OA ratio was not affected by high EC12 mS/cm (Fig. 1 and 2). Tomato plants treated with EC2 mS/cm and EC4 mS/cm produced bigger fruit than EC8 mS/cm and EC12 mS/cm (Fig. 1 and 2). The fruit weight, size decline up to 50% whilst dry matter almost doubled in EC12 mS/cm (Fig. 2). The EC, salt and NO² concentration in culture medium increased by 53.9, 70.3, and 52% at higher EC because of high amount of "onsen" water applied. The high pH of "onsen" water (pH 7.2) did not affect the culture medium pH in high EC. The difference in plant height among salt stress treatment was



Fig. 1 - Effect of different concentration of "onsen" water on fruit SSC Brix%, dry matter and organic acid of tomato plants variety 'Reika' in the greenhouse.



Fig. 2 - Effect of different concentration of "onsen" water on fruit size, weight and NO⁻³ of tomato plants variety 'Reika' in the greenhouse.

observed after about three weeks "onsen" water application. The curve indicates that tomato plants treated with EC2 and EC4 mS/cm had thicker stems and wider leaves than other treatments (Fig. 3). Plants treated with EC2 mS/cm and EC4 mS/cm were the tallest compared to other treatments (Fig. 4). The reduction of plant height, leaf size, and thickness was 12%, 26.8%, and 21.3% respectively in EC12 compared to EC2.

4. Discussion and Conclusions

Salt stressed by "onsen" water showed a significant difference (P<0.0001) on fruit soluble solids,



Fig. 3 - Effect of different concentration of "onsen" water on stem diameter, leaf size and SPAD of tomato plants variety 'Reika' in the greenhouse.



Fig. 4 - Effect of different concentration of "onsen" water on height of tomato plants variety 'Reika' in the greenhouse.

organic acid, NO₃-concentration, and average fruit weight. Massaretto *et al.* (2018) demonstrated that soluble solids content is an important fruit quality parameter which increases depending on the salinity levels. In their study soluble solids contents were significantly higher in both Negro Yeste (60%) and Verdal (78%) landraces compared with Moneymaker (34%) tomato variety under salt stress. In general, there is a negative relationship between SSC and weight in tomato (Higashide and Heuvelink, 2009). Many studies (Adams and Ho, 1992; Cuartero and Fernández-Munaoz, 2001; Cuartero *et al.*, 2006) demonstrated that one of the well-known effects of salinity is the increasing of SSC and antioxidants but with a reduction in the fruit size and weight. In present study, high EC increased the tomato fruit SSC and organic acid while weight and size declined (Fig. 1 and 2). Fruit weight and size reduction under salinity stress was triggered by inhibition of water uptake by the root resulting in insufficient amount of water required for the fruit and increased concentrations of reducing sugars and acids as compared to non-saline conditions. Thus increased concentration of soluble solids was observed in the present study (Fig. 1).

Fruit juice acidity increased with high EC and this could be due to the higher Na content in the fruit juice, since this was the main ion found in "onsen" water. Amjad *et al.* (2014) stated that the accumulation of reducing sugars and organic acids is responsible for increased titratable acidity and decreased pH of the fruit juice. The reduction in fruit water content have caused an increase in fruit dry matter content (Fig. 3). This could be as a result of drought leading to excess amounts of salt in the root zone, which leads to reduced photosynthetic capacity, or toxicity of salt in plant tissues (Amjad *et al.*, 2014).

At the first truss, fruit weight was not significantly influenced by salinity. The reductions started at the second truss and this can be explained by long exposure on salinity.

Considering a reduction in fruit fresh weight (40%) compared with the increase in dry weight (50%), and SSC (50%), the lower water content in the fruits can be pointed as the main reason for the reduction in fruit weight. The accumulation of Na in the soil pot showed by the salt concentration in drained soil solution (from 0.68% to 0.9%) was due to high amount of "onsen" water applied. This contributed to the increased nutrient solution EC (from EC12 to EC16) which probably created an osmotic pressure around the roots. Thus, a reduction of water uptake by plants had resulted by NO₃ concentration in culture medium which increased 1000 ppm to 1600 ppm.

Most of the growth parameters were affected by high salinity treatment. The plant height, stem diameter, leaf size were significantly decreased (Fig. 3 and 4). Bustomi Rosadi *et al.* (2014) mentioned that the plant height was significantly affected by high salinity due to water stress. Ashraf *et al.* (2021) also reported that tomato plants grown under salinity stress in the presence of 200 mg N kg⁻¹ in different NH4⁺:NO⁻³ ratios showed a variable decline in growth in terms of plant height, plant girth, and stem diameter compared to no salinity treatment (respective controls). The reduction in plant growth parameters of our results can be explained by low water potential of the soil solution due to the high EC and salt concentration as it was evident in drained solution. Our results also showed an increase of fruit NO₃⁻ in high EC whereas fruit size and weight were reduced (Fig. 2), this can be attributed to transport of water from the roots to the fruits.The salt stress by "onsen" water in the nutrient solution reduces the ability of the plant to take up water, and this leads to reductions in the growth rate. Results of our experiment indicate that the salt stress by "onsen" water is also effective for increasing the sweetness of tomato fruit. In conclusion, the EC8 and EC12 treatments have the most effective to increase the sweetness of tomato fruit with a reduction in fruit weight.

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