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Growth and physiological responses of coffee (*Coffea arabica* L.) seedlings irrigated with diluted deep sea water

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Concentrations of 5, 10, 20, and 40% deep sea water (DSW) were tested, with irrigation water serving as the 0% control (tap water) on coffee (*Coffea arabica* L.) seedlings. The results showed that the growth parameters were affected significantly ($\alpha < 0.05$) by the irrigation of 20 and 40% deep sea water. There were significant differences ($\alpha < 0.05$) among treatments in stomata density/mm^2, stomata width, and length. The highest value of stomatal measurements was obtained in the control treatment, whereas the lowest values were obtained in the 40% DSW treatments. Electrolyte leakage was enhanced in 20 and 40% DSW irrigated seedling leaves. The highest relative leaf water content (84.5%) was obtained in the control treatment and the lowest in 40% DSW (74.6%). The application of diluted deep sea water also increased the soil electrical conductivity (EC, ds/m). The overall measured parameters indicated that the control, 5, and 10% DSW treatments showed approximate results. This indicates that 5% DSW can be used as irrigation water for coffee seedlings. Also, for some period of time, the 10% DSW can be used to irrigate coffee seedlings without causing significant negative effects.

Key words: *Coffea arabica* L., electrolyte leakage, relative water content, stomata.

INTRODUCTION

Coffee is one of the most important agricultural commodities in the world trade and is considered to be the main income source in developing countries (FAOSTAT, 2008). The world coffee market is dominated by the *Coffea arabica* L. and *Coffea canephora* species, which account for about 99% of the world coffee bean production (Da Matta and Ramalho, 2006). Arabica coffee accounts for about 62% of world coffee consumption and the rest is accounted for by robusta coffee (Morais et al., 2012). In 2016/2017, the global coffee production was estimated at 153.9 million bags, a 1.5% increase on 2015/2016 (ICO, 2017). Arabica production was up by 10.2% to 97.3 million bags, while Robusta was estimated down 10.6% to 56.6 million bags. Currently, climate change is the major threat to coffee production. The availability of quality irrigation water is vital for healthy plant growth and maximize the yield. However, on reclaimed land, saline water can be used for irrigation due to an absence or limited supply of fresh water. In addition, the groundwater used for irrigating

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crops near coastal areas is frequently saline (Lee et al., 2008).

The use of saline irrigation water has adverse effects on soil-water-plant relations, occasionally severely restricting the normal physiological activity and productive capacity of the crops (Plaut et al., 2013). Abiotic stress is one of the serious constraints that limit agricultural production and cause severe yield reductions, such as salinity and drought (Bray et al., 2000). Salinity can affect plant growth in various ways, mainly as the result of toxic ion accumulation in the root zone of plants and through osmotic stress. However, several plants have developed mechanisms to tolerate these effects (Munns, 2002). The evaporation of sea water has created salt and potentially caused soil salinity in adjacent areas since ancient times. Naturally or anthropogenically, a high concentration of soluble salt occurs in terrestrial environments or aquatic environments (Larcher, 1995).

Deep sea water (DSW), generally refers to sea water from a depth of more than 200 m and is estimated at 95% of all the sea water. The use of seawater for agricultural irrigation has been studied for decades due to its high mineral content (Mount and Schuppan, 1978; Feigin, 1985; Glenn et al., 1998; Sgherri et al., 2008). Deep sea water has various trace elements that might be useful to soil lacking them, and it therefore has the potential to stimulate healthy plant growth. The abundant nutrients of deep sea water are also favorable for agriculture. Studying the use of sea water irrigation for the production of agricultural crops can provide a resource to further studies about the use of saline water for irrigation in the areas where there is a limited availability of freshwater resources.

However, the uses and impacts of deep sea water irrigation on coffee plants have not been studied. Therefore, this research was conducted to study the growth and physiological response of coffee seedlings irrigated with different concentration of diluted deep sea water, and thereby to examine the salinity effects.

MATERIALS AND METHODS

Plant and treatments applied

The experiment was carried out under greenhouse conditions at Kangwon National University, Gangwon Province, Korea, during 2016. Six-months-old healthy coffee seedlings were transplanted into small pots (each 12 cm in diameter) that were filled with soil and compost (2:1). The seedlings were well watered and kept in a shaded area so as to create a conducive environment for the transplanted seedlings to become established. The deep sea water was collected from the east sea of Korea at 600 m depth (April, 2016). After that, the water was delivered by 20 L white transparent container and kept in the coffee greenhouse. The applied treatments were different concentrations of diluted deep sea water: control (0.2 DS/m), 5% (2.3 dS/m), 10% (3.6 dS/m), 20% (6.7 dS/m) and 40% (8.1 dS/m). The dilutions were prepared by mixing the deep-sea water with normal irrigation water (tap water) at different concentrations. Finally, the electrical conductivity (EC) of each dilution was measured using an EC meter. The design of the experiment was completely randomized with 3 replications. For this experiment, a total of 15 (n=5, n×3=15) seedlings per treatment were used. Irrigation was started one week after the seedlings had become well established and continued at four-day intervals at a volume of 330 ml/seedling for 3 consecutive months. Uniform agronomic practices were applied to all of the seedlings.

Growth measurements

Measurements of growth were taken for all of the treatments once every 2 weeks. Initial measurements of seedlings' heights (cm), stem diameters (mm), leaf lengths (cm), and leaf widths (cm) were recorded (26/04/2016) and continued until the end of the experiment (26/07/2016). The leaf length and width were recorded from newly developed (top positioned) leaves and continued up to the end of the trial from the same leaves. A caliper (Mitutoyo 530-124 Vernier Caliper) and ruler were used to measure the growth parameters.

Stomata measurements

The coffee leaves were collected from all treatments and prepared for stomata assays. The epidermis from the lower parts of the leaves was peeled using forceps and placed on microscope slides. The staining solution was added to get a clear picture. Image analysis was performed using ImageJ software (https://imagej.nih.gov/ij/) to measure the stomatal length (µm) and width (µm). Thirty stomata per treatment were measured. The number of stomata per unit area (μm^2) was counted and then converted into mm^2 using the formula:

Stomatal density = number of stomata in entire FOV / area (mm^2),

where FOV is the field of view.

The stomata picture was captured by a microscope (Leica, DM 1000; 40x for counting and 100x for size measurement) from all treatments.

Relative leaf water content (%)

The leaf discs were prepared from 3 to 4 leaves to obtain about a 5 to 10 cm^2/sample and immediately weighed to obtain the fresh weight (FW). The samples were immediately soaked in deionized water in a closed Petri dishes to full turgidity for 4 h under normal light and room temperature. After 4 h, the samples were re-weighed to obtain the turgid weight (TW). After that the samples were oven dried at 80°C for 24 h and weighed to estimate their dry weight (DW) of samples. All weighing have been made to the nearest milligram (mg). Finally, the relative water content (RWC) was calculated using the formula:

$$RWC (\%) = ((FW-DW) / (TW-DW)) \times 100,$$

where FW is the sample fresh weight, TW is the sample turgid weight, and DW is the sample dry weight (Barrs et al., 1962).

Relative EC of leaf tissue of coffee seedlings (%)

Fifteen freshly cut leaf discs (0.5 cm^2 each) were prepared from each treatment, rinsed three times (3 min) with demineralized water and soaked in 10 mL of demineralized water. The electrolyte leakage was determined by measuring the EC of the solution (named Initial EC) after 22 h keeping at room temperature, using a

Table 1. The average growth parameters increment of coffee seedlings irrigated with deep sea water (DSW) during the experimental period.

Treatment	Plant height (cm)	Stem diameter (mm)	Leaf length (cm)	Leaf width (cm)
Control	14.3 ± 0.68[a]	1.5 ± 0.39[a]	14.1 ± 2.28[a]	6.1 ± 1.31[a]
DSW 5%	13.0 ± 0.55[ab]	1.3 ± 0.12[b]	13.9 ± 0.40[a]	5.5 ± 0.43[ab]
DSW 10%	11.4 ± 2.44[b]	1.2 ± 0.12[b]	13.0 ± 0.36[ab]	5.3 ± 0.25[b]
DSW 20%	6.7 ± 2.15[c]	0.8 ± 0.15[c]	8.7 ± 1.06[b]	3.2 ± 0.25[c]
DSW 40%	6.1 ± 0.42[c]	0.7 ± 0.52[d]	6.0 ± 1.65[c]	2.0 ± 0.06[d]
Mean	10.41	1.1	11.2	4.4
LSD	1.6	0.1	1.6	0.7
CV (%)	8.7	5.3	7.8	8.7

Results are presented as mean ± standard deviation (n = 3). Values with the same letters within the same columns are not significantly different. CV: coefficient of variation, LSD: list significant differences

conductivity meter (Mettler-Toledo AG-8603). Total EC was obtained after keeping the flasks in an oven (90°C) for 2 h. The results were expressed as % of total conductivity:

REC (%) = (Initial EC/Total EC) × 100

Soil EC (dS/m)

The soil samples were well mixed and 10 g air-dry soil (<2 mm) was weighed from each treatment to prepare a 1:5 soil:water suspension (50 ml of deionized water used). The solutions were mechanically shaken for 1 h at 15 rpm to dissolve soluble salts. The conductivity meter was calibrated according to the manufacturer's instructions using the potassium chloride (KCl) reference solution to obtain the cell constant. Then, the electrical conductivity was measured using a conductivity meter from the soil suspension by inserting the conductivity cell and the value was recorded for each treatment. The conductivity cell was carefully rinsed with deionized water between samples (Rayment and Higginson, 1992). The soil electrical conductivity was measured twice, before the treatments began and after the end of experiments.

Soil pH

The soil samples were taken from all pots (air-dried and passed through a 2-mm sieve) and well mixed. From each sample (25 g), soil was measured and mixed with 40 mL of water (distilled or de-ionized water) to each cup using a suitable volumetric container. The solution was stirred with a glass rod and the sample was allowed to sit for 30 min. The pH meter (Mettler-Toledo GmbH, CH-8603) was calibrated according to the instructions with 2 buffer solutions (pH 4.0 and 7.0). The samples were stirred again immediately before measuring the pH. The electrode was positioned in the solution just above the sand layer. The measurements were repeated 3 times to ensure accurate results. The electrode(s) was rinsed 3 times with de-ionized water after each use and before testing another sample (Hanlon and Bartos, 1993). The soil pH was measured twice, before the treatments began and right after the experiment completed.

Data analysis

ANOVA was used to determine the significance of variance among treatments based on the recorded data. In particular, the growth parameter differences (final data - initial data) during the experimental period were used for statistical analysis. The collected data were subjected to the SAS 9.0 software. The Microsoft Excel (2013) program was used to summarize the data and make a graph.

RESULTS AND DISCUSSION

The response of growth parameters

The results showed that all of the tested deep sea water (DSW) concentrations (5, 10, 20, and 40%) affected the growth and physiological parameters of coffee seedlings in comparison with the control treatment. However, the coffee seedlings that were irrigated with 5 and 10% DSW showed results that more or less approximated those of the control treatment. There were statistically significant differences ($\alpha < 0.05$) among treatments in plant height, stem diameter, leaf length, and leaf width (Table 1).

The highest growth increment in plant height was recorded in the control treatment (14.3 cm) and the lowest in coffee seedlings irrigated with 40% DSW (6.1 cm) (Table 1). This could be because of the high salt concentration present in 40% DSW. These results agree with several researchers who reported that increasing the salt concentration lead to a decrease in leaf area and plant height on bean plant (Mathur et al., 2006; Qados, 2011), sugar cane (Jamil et al., 2007) and oat (Zhao et al., 2007). Yadav et al. (2011) also mentioned that salt has two major effects on plants: osmotic stress and ionic toxicity, both of which affect all plant's primary processes. Moreover, in the present experiment, the results indicated that seedlings irrigated with 20 and 40% DSW showed significantly poor growth due to the effects of salt stress. El-Abagy et al. (2012) reported that in lettuce, salt stress negatively affects plant growth and the production of dry matter. Also, additional reports published about increasing salt concentrations in irrigation water have revealed that the practice may lead to a significant decrease in lettuce growth, yield, marketable yields,

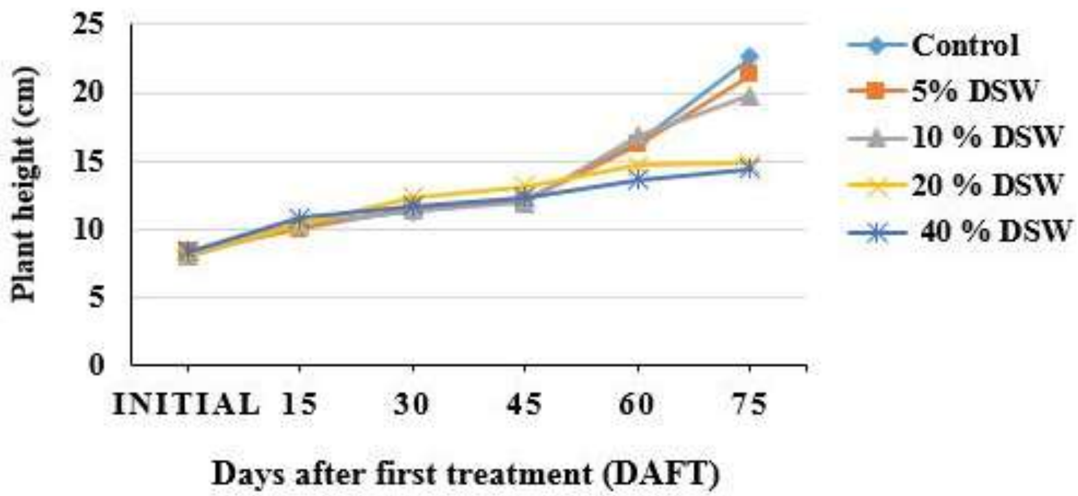

Figure 1. The effect of deep sea water treatments on plant height of coffee seedlings at every two weeks interval.

weight, and the amount of dry matter (Miceli et al., 2003; Mekki, 2007; Al-Maskri et al., 2010).

The increments in stem diameter, leaf length, and leaf width recorded in the control treatment during the experimental period were 1.5 mm, 14.1 cm, and 6.1 cm, respectively (Table 1). According to the results, there was no significant difference ($\alpha > 0.05$) in growth parameters between the control seedlings and those treated with 5% DSW except in the stem diameter. This indicated that the 5% DSW treatment can be used as irrigation water in the area where there is a shortage of fresh water for irrigation. Subsequently, the nutrients that exist in deep sea water will contribute to the growth and development of the plant. Similarly, the differences between 5 and 10% DSW treated coffee seedlings in all growth parameters were not significant. There were significant differences ($\alpha < 0.05$) among treatments in leaf length and width (Table 1). The 20 and 40% DSW treatments greatly decreased the coffee seedling leaf length and width, in comparison to other treatments. This could be because the salt concentration in 20 and 40% DSW presented in a higher amount and affected the leaf area. This leads to a reduction in the photosynthetic area, and therefore affects overall plant growth. This result is supported by Hasanuzzaman et al. (2013). They noticed that salt accumulation in leaves leads to salt toxicity in plants and later on may result in complete leaf death. It also reduces the total photosynthetic leaf area, which reduces the supply of photosynthate (food) in plants and ultimately affects the growth of the plants. Leaf length and width between the control and the 5% DSW treatment did not differ significantly. Generally, the growth performance of the control and 5% DSW treated coffee seedlings were similar. This can be an implication that 5% DSW will be used to irrigate coffee seedlings without causing adverse problems and 10% DSW can also be used to some extent considering application frequency. Frequent application of deep sea water results in an increase of salt concentration in the root zone of the plants.

Data were collected at 2 weeks intervals to study the effects of deep sea water treatment on the growth parameters of coffee seedlings. Similar plant height growth trends were observed in all treatments from the initial treatment application until 45 days after first treatment (DAFT). The similarity continued in control, 5 and 10% treated seedlings until 60 DAFT (Figure 1), whereas the 20 and 40% treated coffee seedlings showed a reduction in plant height growth starting from 45 DAFT in comparison to other treatments (Figure 1). The stem diameter growth in control, 5, and 10% DSW treated coffee seedlings had similar patterns from the initial application time to 75 DAFT. However, the 20 and 40% DSW treated seedlings stem diameter growth was inhibited and the variation became significant towards 45 DAFT, compared to other treatments (Figure 2). Salt stress greatly reduces the size of leaf area. In the present study, the 20 and 40% DSW treated seedlings leaf length and width were reduced after 45 DAFT (Figures 3 and 4). Hasanuzzaman et al. (2013) stated that the time needed to observe the response of plants to salt stress varies according to the species and salinity level. With annual species, the timescale is a day or a week, whereas, with perennial species, the timescale is months or years. However, in this experiment the salt stress effect clearly observed and the growth parameters progress declined in 20 and 40% DSW treated coffee seedlings starting from 45 DAFT.

Stomata size and density

There were significant differences ($\alpha < 0.05$) among

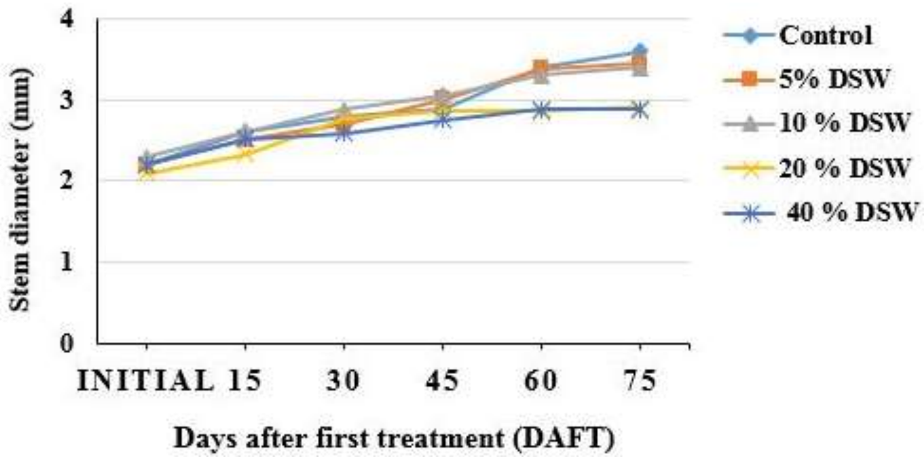

Figure 2. The effect of deep sea water treatments on stem diameter of coffee seedlings at every two weeks interval.

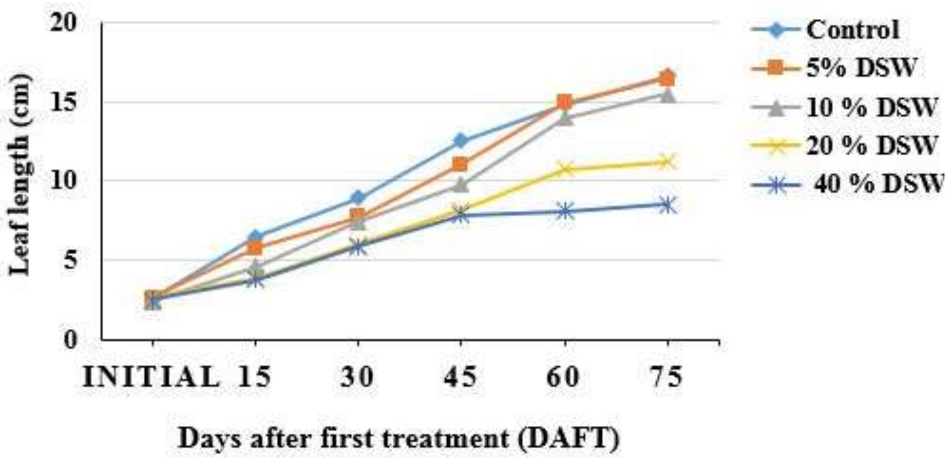

Figure 3. The effect of deep sea water treatments on leaf length of coffee seedlings at every two weeks interval.

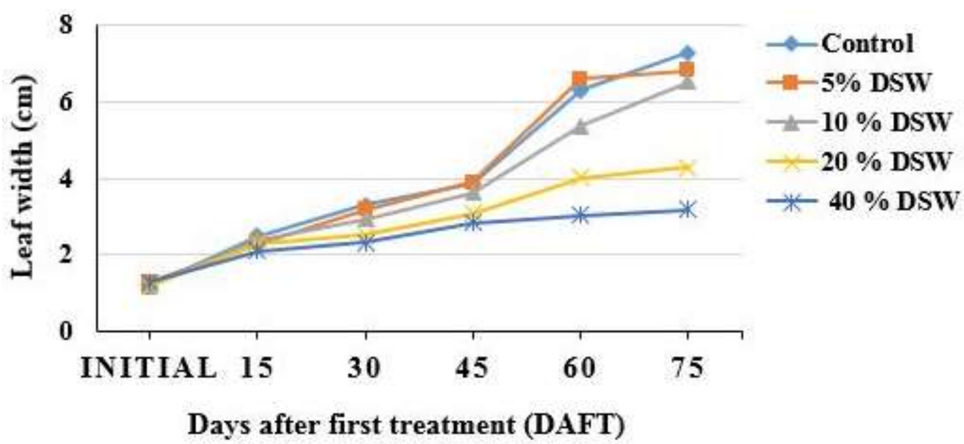

Figure 4. The effect of deep sea water treatments on leaf width of coffee seedlings at every two weeks interval.

Table 2. The stomata length, width and stomata density of coffee seedlings leaves that were irrigated with deep sea water (DSW).

Treatment	Stomata length (µm)	Stomata width (µm)	Number of stomata/mm^2
Control	20.9 ± 0.25^a	17.1 ± 0.36^a	179 ± 14.21^a
DSW 5%	20.3 ± 0.45^{ab}	16.6 ± 0.36^{ab}	173 ± 7.87^{ab}
DSW 10%	20.2 ± 0.39^{ab}	16.1 ± 0.29^{bc}	168 ± 8.80^{ab}
DSW 20%	19.2 ± 0.64^{bc}	15.9 ± 0.61^c	162 ± 10.15^b
DSW 40%	18.8 ±1.62^c	15.4 ± 0.41^c	160 ± 8.49^b
Mean	19.8	16.3	168.4
LSD	1.10	0.61	13.41
CV (%)	4.2	2.6	6.0

Results are presented as mean ± standard deviation (n = 5). Values with the same letters within the same columns are not significantly different. CV: coefficient of variation, LSD: list significant differences.

treatments in stomata length and width. A significant difference ($\alpha < 0.05$) was found between the control and 20% or the control and 40% diluted deep sea water irrigated coffee seedlings, regarded as stomatal density/mm^2. There was no significant difference between the control and 5% DSW treatment in stomata length, width and number of stomata/mm^2 (Table 2). Stomata are used as environmentally controlled gateways into the plants, regulating CO_2 uptake and transpiration. They are also involved in controlling of photosynthesis, nutrient uptake and cooling plants (Farooq et al., 2009). In plant evolution, development of stomata can be considered as a relevant feature of the plant (Brodribb and McAdam, 2011).

The highest stomata length and width have been obtained in control treatment (20.3 and 16.6 µm, respectively) treatment (Table 2). The lowest stomata length and width were recorded in 40% diluted deep sea water (18.8 and 15.4 µm, respectively) treated coffee seedling leaf. The number of stomata decreased as the salt concentration in the treatment (DSW) increased. This result of our experiment is similar to that of Pratima and Cholke (2010), who reported that the number of stomata on the leaves of *Crotalaria* species (namely, *Crotalaria rutusa* and *Crotalaria verrucosa*) decreased as the soil salinity increased. However, the number of stomata in another *Crotalaria* spp. (*Crotalaria juncea*) increased under salt stress conditions. This shows that the stomata distribution of different plant species varies under salt stress. According to Solmaz et al. (2011), the leaf area, leaf size, stomata length, and stomata width of watermelons reduced while the density of the stomata increased under salt stress conditions. The changes in stomata density and size were mainly attributed to changes in leaf area under salt stress (Curtis and Läuchli, 1987) and drought stress (Yang et al*.*, 1995; Chaves et al*.*, 2003; Yin et al*.,* 2006; Gazanchian et al*.,* 2007) conditions. The maximum number of stomata was 173 mm^{-2} in the control treatment, and the lowest was 160 mm^{-2} for the leaf of a coffee seedling irrigated with 40% DSW (Table 2). The openings of the stomata were wider in the control treatment compared with the treatment involving 20% DSW (Figure 5). Abscisic acid (ABA) level rises in the shoot as the plant is exposed to salt stress, which helps the stomata to close, decreases water loss, and transports transpirational sodium chloride (NaCl) into the shoot (Jaschke et al., 1997; Albacete et al., 2008). However, stomata closure under salt stress conditions also significantly affects the intake of CO_2 for photosynthesis.

Relative water content of leaves (%)

There were significant differences ($\alpha < 0.05$) among treatments in the relative water content (RWC) of leaves. The highest RWC was determined in the control (84.5%) treatment, whereas the lowest in 40% DSW (74.6%) irrigated coffee seedling leaves (Table 3). The result showed that as the rate of the DSW concentration increased the RWC of the leaves was decreased. This result is in line with the findings of Shaheen et al. (2013), who reported that salt stress significantly affected the relative water content of the plant. Salt treated plants often show a considerable reduction in the water uptake, which results in a decline in the water content of the various parts including the leaves (Colmer et al., 1995; Curtis and Läuchli, 1987; Machado et al., 2017). However, the RWC of leaves in control (84.5%), 5% (82.6%) and 10% (80.7%). DSW irrigated coffee seedlings, did not differ significantly ($\alpha > 0.05$) (Table 3).

Relative EC of leaf tissue of coffee seedlings (%)

Electrolyte leakage was significantly enhanced as the deep sea water concentration increased compared to the control treatment. The highest EC% obtained in 40% DSW treated coffee leaves (95%) and the lowest found in the control treatment (~0%). The electrolyte leakage of 5

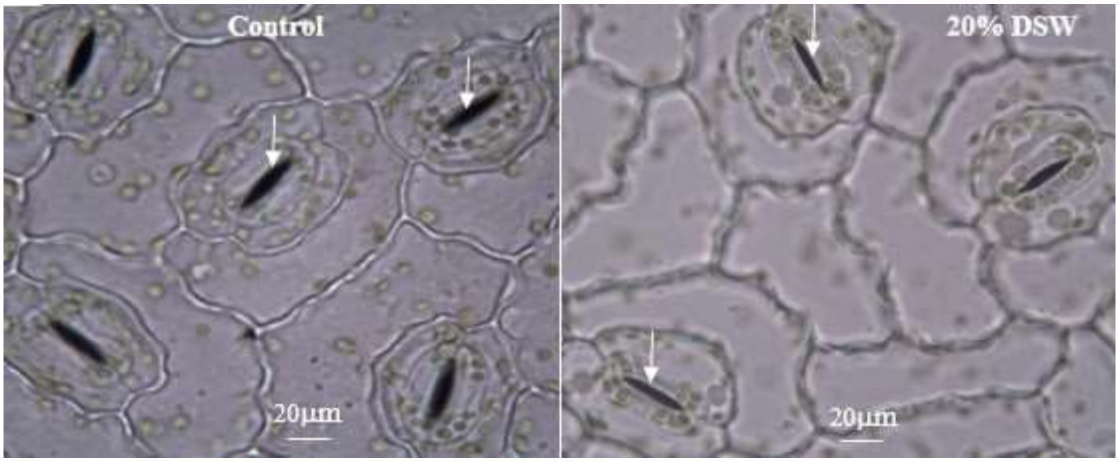

Figure 5. Leaf stomata from control and deep sea water (20% DSW) treated coffee seedlings (Control: stomata were opened widely; 20% DSW: stomata were opened narrowly than control treatment and at the same time the number of stomata in 20 % DSW treated seedling leaf were fewer than that of in the control treatment).

Table 3. The relative water content (RWC %) of coffee seedling leaves that were irrigated with deep sea water (DSW).

Treatment	RWC (%)
Control	84.5 ± 1.33a
5% DSW	82.6 ± 3.10ab
10% DSW	80.7 ± 2.37ab
20% DSW	80.1 ± 2.75b
40% DSW	74.6 ± 1.80c
Mean	80.5
LSD	4.3
CV%	3.32

Results are presented as mean ± standard deviation (n = 3). Values with the same letters within the same column are not significantly different. CV: coefficient of variation, LSD: list significant differences, RWC: relative water content.

and 10% DSW treated coffee seedling leaves were similar (14%) and the 20% DSW treated resulted in 35% (Figure 6). Several researchers reported that an increase in electrolyte leakage as plants were exposed to salinity (Dkhil and Denden, 2012; Kaya et al., 2001a, b). In this experiment also the higher electrolyte leakage was obtained due to the salt stress effect.

Soil EC (dS/m) and pH

For both soil parameters (EC and pH), we used the final data that were recorded right after the end of the experiment for statistical analysis, since the initial data were similar from all experimental pots soil. Application of deep sea water significantly increased the soil EC (Figure 7). The soil EC (dS/m) increased as the DSW concentration raised. The result agrees with the findings of Huang et al. (2011) who mentioned that the soil EC values increased as the concentration of saline irrigation water increased. The highest soil EC obtained in 40% DSW irrigated soil, and the lowest was in the control (8.97 and 2.0 dS/m, respectively) (Figure 7). The result is in line with the findings of Chadirin et al. (2008), who reported that the soil EC increased after the DSW treatment applied in tomato experiment. The 5, 10 and 20% DSW irrigated soil EC were, 5.1, 7.07 and 7.77 dS/m, respectively (Figure 7).

According to the soil salinity classification, non-saline soil EC ranges between 0 and 2 dS/m which is similar to the result of control treatment (2.0 dS/m) in this study. The other 3 treatments (5, 10 and 20% DSW) categorized under the moderately saline soil and 40% DSW irrigated soil classified under severely saline soil.

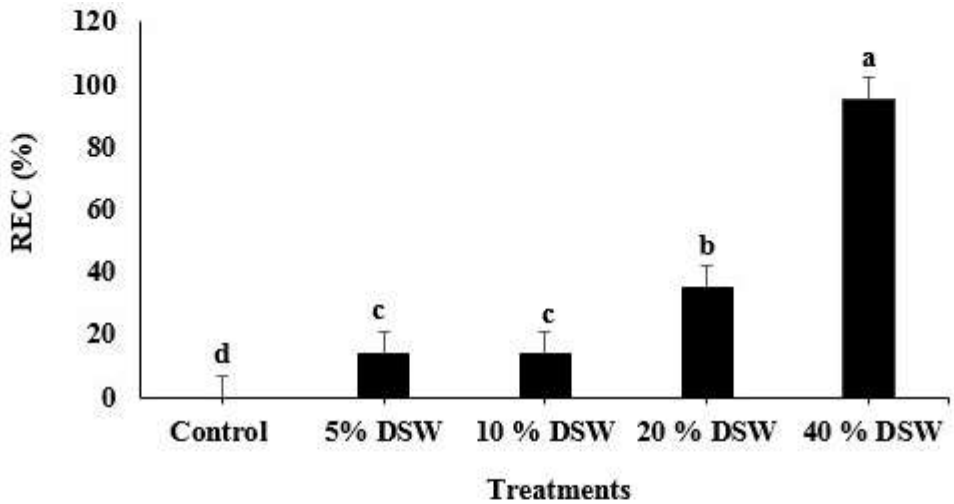

Figure 6. The relative electrolyte leakage of coffee seedling leaves irrigated with deep sea water. The vertical bars represent the means (n = 3). The bars with different letters indicate a significant difference ($p < 0.5$) among treatments. DSW: Deep sea water. REC: relative electrical conductivity.

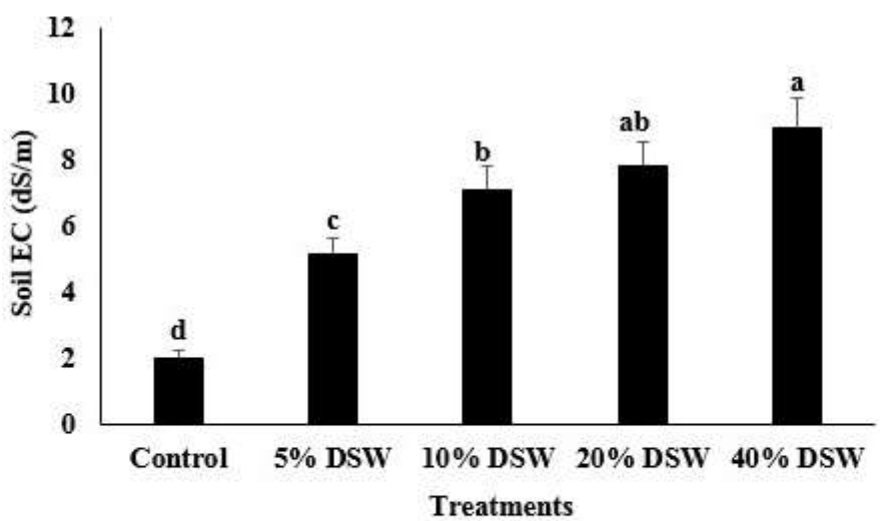

Figure 7. The effect of deep sea water irrigation on soil electrical conductivity. The vertical bars represent the means (n = 3). The bars with different letters indicate a significant difference ($p < 0.5$) among treatments. DSW: deep sea water.

The application of deep sea water during the experiment period did not significantly affect the soil pH. The soil pH was in the moderate range (5.6-6.0) (Figure 8).

Conclusion

The results indicate that all the tested diluted deep sea water concentration with a continuous four-day irrigation interval affects the growth and physiological parameters of coffee seedlings and other relevant parameters in comparison with the control treatment. However, an approximate result was obtained from the control, 5 and 10% DSW irrigated coffee seedlings. This indicates that 5% DSW can be used as irrigation water for coffee seedlings. For some period of time, 10% DSW also can be used to irrigate coffee seedlings without causing significant negative effects on their growth and physiological activities. Further investigation is crucial to understanding the optimum concentration of diluted deep

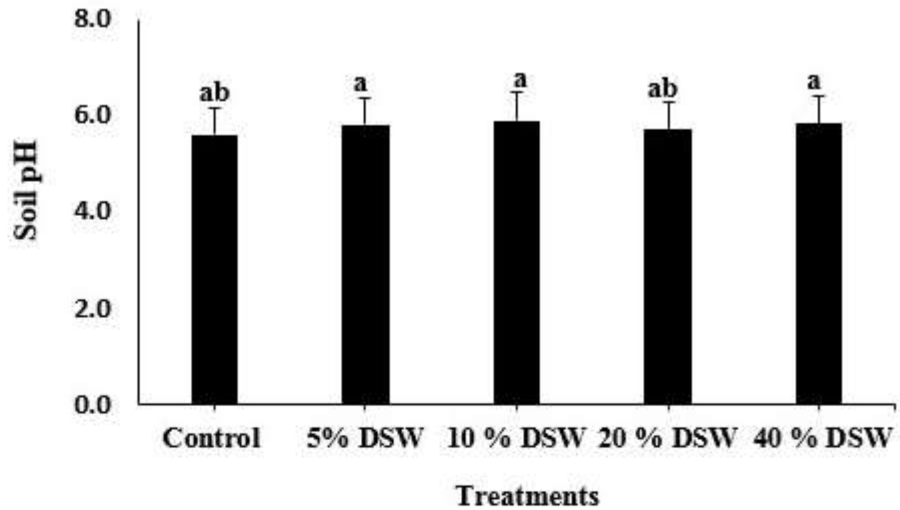

Figure 8. The effect of deep sea water irrigation on soil pH. The vertical bars represent the means (n = 3). The bars with different letters indicate a significant difference ($p < 0.5$) among treatments. DSW: Deep sea water.

sea water and application interval. The frequent use of diluted sea water increases the salt concentration in the root zone of the plants. Instead of the continuous use of diluted deep sea water, reducing the rate and the frequency of application will have better results in improving the growth and development of coffee seedlings.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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