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# Morpho-physiological Response of Avocado (*Persea americana* Mill.) Seedlings to Different Salinity Levels in Sidam National Regional State, Southern Ethiopia

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**Abstract**

*Salt stress is the most important environmental stress that adversely affects avocado morpho-physiological performance. Therefore, this study has been conducted to evaluate the response of avocado seedlings to different salinity levels. The experiment was laid out in a Randomized Complete Design (RCD) with four salt concentration levels (0, 1.3 dS/m, 2.6 dS/m, and 3.9 dS/m) on grafted avocado seedlings, with three replications. The avocado seedlings were raised from Ettinger avocado scion grafted on Guatemalan race rootstock. The results indicate that most of the morphological growth parameters, such as rootstock diameter, sucker development, and root length, were significantly (p<0.01) affected by salt stress level. While tap root diameter and lateral root length were not significantly (p>0.05) affected by salt treatment. Except for the number of suckers developed on the rootstock, rootstock and scion diameter significantly decreased as salt concentrations increased from 0 to 3.9 dS/m. Similarly, significant (p<0.01) variations were also observed in leaf length, bud number, leaf fresh weight and dry weight, leaf area, shoot height, taproot length, and lateral root length. These parameters were also significantly decreased with the increase in salt concentrations. Likewise, photosynthesis, transpiration, stomatal conductance, and instantaneous water use efficiency were significantly affected by salt concentration, where relatively maximum scores were obtained at 0 and 1.3 dS/m. In general, salt concentration at 0 and 1.3 dS/m EC favors most of the growth and physiological performance of avocado seedlings compared to 2.6 and 3.9 dS/m. Thus, the study revealed that for vigorous growth of avocado seedlings at an early stage, the quality of irrigation water needs considerable attention to ensure robust avocado seedling growth.*

**Keywords/Phrases**: Grafting, Photosynthesis, Rootstocks, Salt, Stress

## 1 Introduction

Avocado (*Persea americana* Mill.) is native to Mexico and Central America, belonging to the family Lauraceae, and is one of the most economically important tree crops in the world (Silva and Ledesma, 2014). It has numerous advantages, particularly in terms of nutritive value (rich in monounsaturated fatty acids (MUFA), dietary fiber, essential nutrients, and phytochemicals (Fulgoni *et al*., 2013)); it also functions as shade trees, windbreaks, and ornamentals (Albertin and Nair, 2004). In the last century, it has been distributed to more than 50 countries around the world, including sub-tropical and tropical regions, with Ethiopia identified as one of the top five avocado producers in tropical Africa.

In Ethiopia, despite its long history since introduction and the presence of suitable diverse agro-ecological conditions (Wayessa and Berhanu, 2010), its distribution was still limited to a few areas of the country (Derebe *et al*., 2023; CSA 2014). However, in the last few years, with the development of Agro-Industry Parks and the emergence of direct fruit exports, the expansion to the central, southern, southwestern, and eastern parts of the country has improved. The Sidama region in the southern part of the country is known for its traditional coffee-enset-fruit-based agroforestry farming system (Asfaw and Lemenih, 2010) and is also where avocado was first introduced in the country (Derebe *et al.*, 2023; Megersa and Alemu, 2013).

Environmental stresses are the most limiting factors in exploiting the potential of horticultural crops worldwide (Celis *et al.*, 2018). Among these, salinity, temperature, nutrition, light, oxygen availability, and metal ion concentration are the most determinant factors. Salt stress is one of the adverse environmental factors influencing physiological processes by altering the osmotic conditions in and around the plant's environment (Jouyban, 2012). High salt concentrations in the external solution of plant cells can cause various effects, which can be demonstrated in three different types: osmotic drought, toxicity due to high chloride and sodium retention, and nutritional imbalance (Karimi *et al.*, 2005).

To overcome these challenges, developing varieties that are tolerant to such stress conditions deserves considerable attention. Breeding for salt-tolerant varieties can help optimize the productivity of horticultural crops in areas affected by high soil salinity or saline irrigation water.

Tolerance to saline irrigation water is emerging as a very important quality for avocado rootstocks as it is one of the most salt-sensitive crops (Grieve *et al.*, 2012). Plants have different mechanisms to withstand saline condition. These are classified (Roy *et al.*, 2014) in to three main categories that is regulated by long-distance signals that reduce shoot growth and is triggered before shoot $Na^+$ accumulation; ion exclusion, during which $Na^+$ and $Cl^-$ in the roots reduce the accumulation of toxic concentrations of $Na^+$ and $Cl^-$ within leaves; and, last, tissue tolerance, in which high salt concentrations are found in leaves but are compartmentalized at the cellular and intracellular levels (especially in the vacuole) (Celis *et al.*,2018 ; Reints *et al.*, 2020).

The toxicity due to salinity increases $Cl^-$ and $Na^+$ concentration. Mickelbart *et al.* (2007) identified that the "relative tolerance of the various rootstocks appeared due primarily to their ability to exclude $Na^+$ and $Cl^-$ from the scion." They also reported that rootstock tolerance ability is indirectly observed from net $CO_2$ assimilation, chlorophyll concentration, and leaf necrosis.

On the other hand, Liao *et al.* (2022) found that mild water and salt stress improved intrinsic water use efficiency (iWUE = A/gs) by (i) decreasing gs via increasing osmotic adjustment and hydraulic resistance, and (ii) declining A via increasing stomatal limitations rather than reducing photosynthetic capacity.

Furthermore, Musyimi *et al.* (2007) reported that salinity significantly affects plant overall growth, net photosynthetic rate (PN), stomatal conductance (gs), transpiration rate (E), water use efficiency (WUE) and chlorophyll (chl) concentration. As the salt concentration increases, it inhibits the growth and gas exchange of avocados.

Concurrently, Celis *et al.* (2018) also reported that rootstocks in the salt-treated soils showed extensive leaf burn. Similarly, "tip-burn" on the leaves in the fall is a sign that the tree is absorbing too much chloride, and these leaves will have to drop off during the winter. Leaves that grow to replace these often grow at the expense of flowering and fruit set, resulting in chronically low yields in some orchards.

Rootstocks vary in their ability to absorb and transport chloride and sodium. In this regard, Guatemalan rootstocks are generally intermediate in their tolerance to salinity. However, only limited research has been conducted on the comparative salinity tolerance of avocado rootstock races. Hence, identifying the performance of avocado seedlings at the early development stage under different salinity levels is highly significant in deciding on the quality of irrigation water and soil media used at the nursery. In Ethiopia, information on the response of avocado seedlings to salinity conditions is very limited. Therefore, this research is conducted to evaluate the morpho-physiological response of grafted avocado seedlings to different salinity levels at Sidama Region, Southern Ethiopia.

## 2 Materials and Methods

### 2.1 Description of the Study Area

The Study was conducted from September 2021 to October 2022 at a Dara district Tafari-Kella site in Sidama National Regional State (Figure 1); located at 350 km south of Addis Ababa at altitudes of 1850 m.a.s.l; and laid at 6°30’0"N latitude and 38°24’0"E longitude. The area receives an average of 1700 mm annual rainfall and 27$^{o}$C average temperatures. The area is well characterized by a coffee fruit farming system where avocado is the dominant fruit crop in the area.

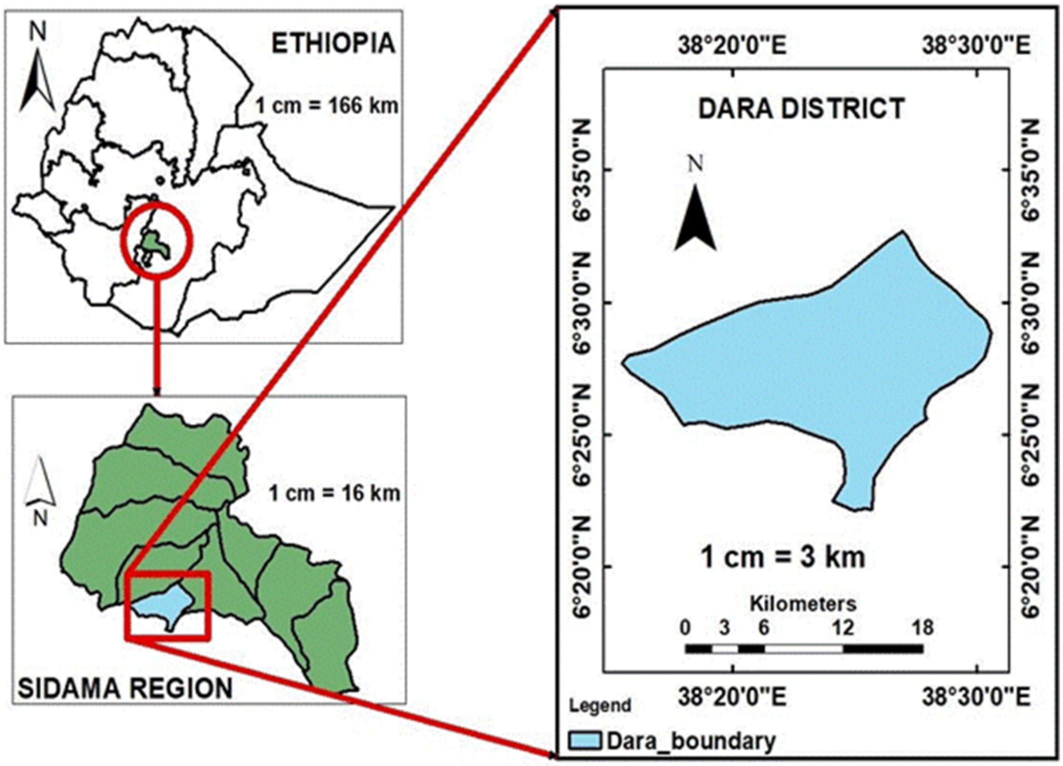


**Figure 1.** Map of the study area (Dara district Tafari- Kella kebele Sidama National Reginal State, Ethiopia)

### 2.2 Experimental Materials and Treatments

The avocado seedlings used as experimental material were obtained from Ethinger avocado scion grafted on Guatemalan race rootstock. The salt treatments were prepared from dissolved salt (*NaCl*) (Table 1) concentrations (1.3, 2.6, and 3.9 dS/m) dissolved in distilled water in separate containers to attain the respective salt concentration based on the following formula: Water Salinity in ECw (dS/m) x 640 = TDS (mg/l)

**Table 1.** List of salt treatment and its formulation from *NaCl*

| Treatment | Salt level ds/m (ECw) | TDs (mg/l) | TDs (g/l) |
|---|---|---|---|
| 1 | 0 ds/m | 0 *NaCl* mg/l | 0g/l |
| 2 | 1.3ds/m | 1.3x640 *NaCl* mg/l=832 mg/l | 0.832g/l |
| 3 | 2.6ds/m | 2.6x640 *NaCl* mg/l=1664 *NaCl* mg/l | 1.664g/l |
| 4 | 3.9ds/m | 3.9x640 *NaCl* mg/l=2496 mg/l | 2.496g/l |

PWhere: ECw=electrical conductivity of water, dS/m= deciSiemens per meter,
P mg/L=milligrams per liter and TDs=total dissolved salt

### 2.3 The Climatic Conditions of shade house

The daily maximum and minimum air temperatures for the shaded house were recorded on five selected days during the experimental period using the temperature and relative humidity data logger "testo". In that case, it was hung close to the seedling canopy. Accordingly, the mean maximum and minimum air temperature in the shade house was 11.7$^o$C and 32.4$^o$C, with a mean minimum and maximum relative humidity of 44.90% and 99.00%, respectively, at the time of the experiment.

### 2.4 Experimental Design

The experiment was conducted under plastic shade in 16 x 20 cm pot size that was filled with a composite soil consisting of a 3:2:1 ratio of topsoil, compost, and sand, respectively. The experiment was laid out in a Completely Randomized Design (CRD) with three replications. Each experimental unit consists of 4 pots per plot.

The four levels of salt treatments (0, 1.3, 2.6, and 3.9 dS/m) were applied after grafting throughout the growth period in the nursery. The treatments were applied once a week, while the pots were irrigated at two-day intervals throughout the experimental period to ensure available water in the root zone.

### 2.5 Data Collection

#### 2.5.1 Physico-Chemical Properties of the Experimental Soil

To determine the physicochemical properties of the soil used for the experiment, a soil sample was collected from the mixed soil prepared for planting. The composite sample was dried and ground to pass through a 0.2 mm sieve before laboratory analysis. The samples were analyzed for parameters relevant to the study, such as pH, texture class, organic carbon, total nitrogen, available phosphorus, CEC, FC, and EC (Table 1), following standard procedures at the Hawassa soil laboratory.

#### 2.5.2 Growth and morphological data

Growth and morphological data were collected from the aerial parts of the plant, such as rootstock diameter, number of suckers, scion diameter, leaf number, number of buds on scion, leaf length, grafted success, rootstock diameter, stem diameter, plant height, and scion diameter. Data collected from the root parts included root length, tap root diameter, leaf fresh weight, stem fresh weight (g), leaf dry weight (g), root dry weight (g), leaf area (cm$^2$), and total fresh and dry weight. All data were collected following standard procedures (Ndoro *et al.*, 2018).

#### 2.5.3 Stomata anatomy

Stomata anatomy measurement was made using the protocol proposed by Xu and Zhou (2008) at 120 days after the treatment was applied. The stomata print was examined using an Automated Upright Leica Microscope DM5000 B with a 40x magnification lens fixed with a digital Leica DFC425/DFC425C image processing camera. For each sample, the following parameters were recorded: stomata number (per mm$^2$), stomata cell number (per mm$^2$), stomata opening ($\mu$m), and stomata length ($\mu$m).

#### 2.5.4 Gas exchange and physiological parameters

Photosynthesis (A, $\mu$mol m$^{-2}$ s$^{-1}$), transpiration rate (E), stomatal conductance (gs), and water use efficiency were estimated from three randomly selected seedlings, using the third young and fully expanded leaves. These measurements were made using a CIRAS-3 portable photosynthesis system (CIRAS-3 PP System Inc., Lincoln, NE, USA). The measurements were taken at 45 days after the actual moisture treatment was imposed on fully developed, intact leaves.

The measurements were done between 9:30 AM and 12:30 PM, with the following specifications maintained: leaf surface area of 6.25 cm$^2$, ambient $CO_2$ concentration of 386 $\mu$mol mol$^{-1}$, leaf chamber mass flow rate of 251 $\mu$mol s$^{-1}$, atmospheric pressure of 840 bar, and manually fixed photosynthetic active radiation (PAR) at 600 $\mu$mol m$^{-2}$ s$^{-1}$.

### 2.6 Statistical Analysis

All morphological and physiological data were subjected to ANOVA following the standard procedure of the General Linear Model (GLM) (Gomez and Gomez, 1984). The statistical analysis was performed using SAS statistical software version 9.0 (SAS, 2008). Mean separation was conducted at a

5% level of significance using the Least Significant Difference (LSD) test.

## 3 Results and Discussions

### 3.1 Physico-Chemical Properties of the Experimental Soil

The physicochemical properties of the soil sampled from the experimental site are presented in Table 2. The results indicated that the soil has a sandy loam texture, with 75% sand, 20% silt, and 5% clay (Clingensmith *et al*., 2022). Additionally, the soil has a medium CEC (33 Meq/100g soil), low EC (0.012 dS/m), 3.47% organic carbon, 10.8 ppm available phosphorus, and a slightly acidic pH of 6.5. These characteristics describe the composition of the soil media used for the pot experiment.

**Table 2.** Physico-chemical characteristics of the soils media composition used in the pot experiment

| Soil properties | Values obtained |
|---|---|
| Sand% | 75 |
| Silt% | 20 |
| Clay% | 5 |
| Textural class | Loam sandy |
| pH | 6.4 |
| Organic Carbon (%) | 3.56 |
| Total Nitrogen (%) | 0.19 |
| Available Phosphorus (ppm) | 10.9 |
| Field Capacity (FC=v/v %) | 31.72 |
| CEC (meq/100g soil) | 33 |
| EC(ds/m) | 0.012 |

### 3.2 Effect of salinity level on growth and Morphology of avocado seedlings

The analysis of variance revealed that rootstock diameter and the number of suckers developed on the rootstock were significantly ($p<0.05$) affected by the salt stress levels, while scion diameter was not significantly affected.

The rootstock diameter was significantly reduced as the concentration of salinity increased. Previous reports have confirmed that avocado plants subjected to higher concentrations of soil salinity experience significantly reduced vegetative growth compared to control conditions (Celis *et al*., 2018). The maximum rootstock diameter (5.9 mm) was recorded from the control (0 dS/m) salt concentration, which was 21% greater than the treatment receiving the maximum salt concentration (3.9 dS/m).

On the other hand, the maximum number of suckers developed at the maximum salt stress level (3.9 dS/m), which was 70% more than the number of suckers developed on the control treatment (Table 3). This may be due to increased metabolic activity in the rootstock part compared to the scion part, as observed in similar studies by Rana and Bhatia (2004) and Lazare *et al*. (2021), in which a wide range of rootstock responses to salinity, including changes in circumference/diameter, were reported.

Consistent with these findings, Vazifeshenas *et al*. (2009) also reported that the number of suckers grown in pomegranate varied based on the genotype. However, no significant variation was observed in scion diameter among the different salinity levels (Table 3).

**Table 3.** Morphological Response of avocado seedlings to different salinity levels

| Treatment Salinity level (ds/m) | Rootstock diameter (mm) | Scion diameter (mm) | No sucker growth ($np^{-1}$) |
|---|---|---|---|
| Control | $5.9^a$ | 5.8 | $0.6^c$ |
| 1.3 | $5.4^b$ | 5.5 | $1.0^b$ |
| 2.6 | $4.7^c$ | 5.2 | $1.5^ab$ |
| 3.9 | $4.3^c$ | 5.0 | $2.0^a$ |
| LSD (p<0.05) | 0.3 | ns | 0.7 |
| CV (%) | 3.94 | 8.72 | 29.5 |
| P=value | 0.006 | 0.31 | 0.01 |

Means with different letter with in the same column are significantly different at (p<.05)

### 3.3 Bud Number, Leave Number and Leaf length

The number of new buds, leaves, and leaf length developed per avocado scion were significantly influenced by the concentration of salinity (Table 4). The highest number of new buds per scion (3.06) was recorded from plants treated with 1.3 dS/m salinity level, while the minimum number of new buds (1.6) was recorded from plants receiving 3.9 dS/m (Table 4). As the salinity concentration increased, the number of new buds developed per scion was significantly reduced.

Salt concentration also significantly influenced the number of leaves developed on avocado scions. Plants exposed to 1.3 dS/m salinity level produced the highest number of leaves per scion, compared to those exposed to 3.9 dS/m salt concentration.

Similarly, different levels of salinity concentration in the growing media significantly influenced the leaf length per avocado scion. The longest leaf lengths of 5.4 cm and 5.3 cm were recorded from plants subjected to 1.3 dS/m and the control (0 dS/m), respectively (Table 4), while the minimum leaf length (2.3 cm) was recorded from plants subjected to 3.9 dS/m (Table 4). This suggests that as the salt concentration increased in the growing media, most of the growth parameters were significantly reduced.

The highest numbers of leaves were recorded from the 0 and 1.3 dS/m treatments, while the lowest numbers of buds on the rootstock were recorded from the rootstock receiving the maximum salt treatment (Table 3). This could be due to the low water uptake, leading to decreased stomatal conductance and other physiological activities, which resulted in reduced leaf growth. Previous studies have reported that the highest number of leaves might be due to photosynthetic accumulation in newly grafted plants, which in turn increased the number of nodes and the absorption of moisture and nutrients by leaf primordia (Mandal *et al.*, 2012; Pampanna and Sulikeri, 1995).

**Table 4.** Effect of salinity level concentration on morphology of avocado (Guatemalan race) scion after grafted on seedling rootstock

| Avocado (GT) | Parameter | | |
|---|---|---|---|
| Treatment (Salinity level (ds/m) | Number of bud ($np^{-1}$) | Leaf number ($p^{-1}$) | Leave length (cm) |
| Control | $3.04^a$ | $5.20^ab$ | $5.36^a$ |
| 1.3 | $3.06^a$ | $5.70^a$ | $5.40^a$ |
| 2.6 | $2.25^b$ | $4.30^b$ | $3.86^b$ |
| 3.9 | $1.67^c$ | $1.83^C$ | $2.10^b$ |
| LSD (p<0.05) | 0.76 | 1.37 | 1.08 |
| CV (%) | 11.58 | 18.14 | 15.1 |
| p-Value | 0.009 | 0.0001 | 0.002 |

Means with different letters within the same column are significantly different at ($p \leq 0.05$)

### 3.4 Stem diameter, plant height, scion diameter, and graft success

Stem diameter, plant height, scion diameter, and grafted success percentage on the rootstock were significantly ($p<0.05$) affected by salt stress levels. The plant height was significantly reduced as the concentration of salinity increased.

The highest plant height (22.6 cm) was recorded from the 1.3 dS/m salt concentration, followed by 21.2 cm and 20.33 cm from the control (0 dS/m) and 2.6 dS/m salinity, respectively (Table 5). The lowest plant height (18.33 cm) was recorded from the seedlings that received the maximum salt concentration (3.9 dS/m). These results indicate that excess salt concentration poses adverse effects on plant growth and development.

Previous studies have revealed that plant growth is sensitive to saline conditions, and the level of sensitivity varies based on the species in higher plants (Bernstein *et al.*, 2001). Berkessa (2020) and Bernstein *et al.* (2004) have also confirmed that avocado plants subjected to higher soil salinity levels showed a significant reduction in plant height, which might be a result of several physiological reactions, such as modification of ion balance, change in water status (water potential) in and outside the plant cell, mineral nutrition processes, and other abnormal metabolic processes.

Reduced plant height under salinity is also associated with reactive oxygen species (ROS); as the ROS are extremely reactive and, in high amounts, noxious, leading to destructive processes and causing cellular damage (Kesawat *et al.*, 2023). Previous studies have also widely indicated that rootstocks are known to influence the salt tolerance of grafted fruit trees, including avocado (Cooper, 1951; Haas, 1950). Similarly, shoot (scion) leaf production in avocado decreases with increasing salinity (Oster and Arpaia, 1992), with differences among rootstocks influencing the extent of growth reduction under stress.

The effect of salinity was also observed on grafted success, where the highest grafted success (99.67%) was recorded from the control (0 dS/m), and 99.3% graft success was from 1.3 dS/m, followed by 43.3% and 25% graft success at 2.6 dS/m and 3.9 dS/m salinity levels, respectively (Table 4). Similar reports by Sibole *et al.* (2003) have revealed that plants subjected to salt stress decrease in growth, as it is directly associated with a reduction in photosynthetic capacity.

**Table 5.** Effect of salinity level concentration on morphology of avocado (Guatemalan race) scion after grafted on seedlings rootstock

| Treatment | Parameter | | | |
|---|---|---|---|---|
| salinity level ds/m | Stem diameter (mm) | Plant height (cm) | Scion length (cm) | Grafted success (%) |
| Control | 21.3 | $21.1^{a}b$ | $11.6^{a}$ | $99.6^{a}$ |
| 1.3 | 22.0 | $22.6^{a}$ | $11.6^{a}$ | $99.3^{a}$ |
| 2.6 | 21.1 | $20.3^{a}b$ | $8.5^{b}$ | $43.3^{b}$ |
| 3.9 | 20.0 | $18.3^{b}$ | $7.9^{c}$ | $25.0^{c}$ |
| LSD (p<O.05) | 3.3 | 2.6 | 0.7 | 1.2 |
| CV (%) | 8.9 | 7.5 | 9.61 | 2.8 |
| P Value | 0.1623 | 0.0194 | 0.0002 | 0.0019 |

### 3.5 Sucker development, Scion diameter and rootstock diameter

The highest scion diameter of 2.1 mm was recorded from the treatment that received 0 dS/m salt, and the minimum scion diameter was recorded in the 3.9 dS/m salt-treated seedling (Table 6).

The highest number of suckers (1.9 per plant) was recorded in the 3.9 dS/m salt-treated plants, while the minimum sucker number was recorded at the control/0 dS/m salt concentration. This may be due to the increased metabolic activity in the rootstock as a result of stress hormone development under high salt concentration, which enhances the metabolic activities of stored food in the rootstock part.

This result is similar to the findings of Rana and Bhatia (2004), Bhatia and Kumar (2011), and Castro *et al.* (2009), who reported an increase in rootstock diameter under salt stress conditions.

On the other hand, Bonomelli *et al.* (2018) reported avocado tree vegetative growth stimulation under low salinity levels in Mexican plants, which they attributed to a rise in cellular growth and an increase in cell number as a result of osmoregulation. This was also supported by Penella *et al.* (2016), who revealed that salt stress significantly stunted plant growth (-40.6% of leaf dry weight) compared to the control conditions.

**Table 6.** Effect of salinity concentration on morphology of avocado (Guatemalan race) after grafted on seedling rootstock

| Avocado (GT) | Parameter | | |
|---|---|---|---|
| Treatment Salinity level (ds/m) | Average sucker growth ($np^{-1}$) | Scion diameter (mm) | Rootstock diameter (mm) |
| Control | $1.10^{b}$ | $2.17^{a}$ | $20.80^{ab}$ |
| 1.3 | $1.16^{b}$ | $2.16^{a}$ | $22.00^{a}$ |
| 2.6 | $1.86^{a}$ | $1.60^{ab}$ | $16.00^{b}$ |
| 3.9 | $1.96^{a}$ | $1.45^{b}$ | $15.33^{c}$ |
| LSD ($p \leq 0.05$) | 0.5 | 0.59 | 1.6 |
| CV (%) | 17.85 | 16.2 | 4.58 |
| P-Value | 0.0145 | 0.0513 | 0.0001 |

Means with different letter with in the same column are significantly different at ($p \leq 0.05$)

### 3.6 Root number, Root length, and Tap and Lateral diameter

Among the root parameters considered, root number per plant was significantly ($p<0.05$) affected by salinity. However, root length, tap root diameter, and lateral root diameter were not significantly affected due to salt treatments (Table 7).

The highest root number (22.4 roots per plant) was recorded from the control treatment, while the minimum root number per plant (9.9) was recorded at 3.9 dS/m. It was also observed that the number of roots decreased with increasing salinity levels (Table 7). A similar result was also reported by Aydinsakir *et al.* (2015).

Although root length, tap root diameter, and lateral root diameter were statistically non-significant ($p>0.05$), the root length increased from 3.74 to 5.48 cm (by 18.9%) as the salt concentration increased from 0 to 3.9 dS/m. On the other hand, the tap and lateral root diameters decreased from 4.9 to 3.9 mm and from 1.09 to 0.65 mm, respectively, as the salt concentration increased from 0 to 3.9 dS/m (Table 7).

**Table 7.** Effect of salinity level solutions on root of avocado seedlings rootstock (Guatemalan race)

| Treatment | Parameter | | | |
|---|---|---|---|---|
| Salinity level (ds/m) | Root number | Root length (cm) | Taproot diameter (mm) | Lateral root diameter (mm) |
| Control | $22.44^{a}$ | 3.74 | 4.94 | 1.09 |
| 1.3 | $16.11^{b}$ | 4.15 | 4.20 | 1.00 |
| 2.6 | $11.33^{b}$ | 4.44 | 4.40 | 0.77 |
| 3.9 | $9.97^{b}$ | 5.48 | 3.90 | 0.65 |
| LSD ($p \leq 0.05$) | 6.3 | Ns | ns | Ns |
| CV (%) | 22.47 | 17.13 | 17.3 | 26.6 |
| P-Value | 0.0074 | 0.18 | 0.4 | 0.15 |

Means with different letter within the same column are significantly different at ($p \leq 0.05$)

It is well-known that plant height and root length are the most important parameters for salinity because roots are in direct contact with the soil and absorb water from the soil, while the shoots supply water to the aerial parts of the plant (Jamil and Rha, 2004). Generally, inhibition of shoot and root development is the primary response to stress. Growth, morphology, anatomy, and physiology of roots are affected by salinity. Changes in water and ion uptake by the roots, production of hormonal signals that communicate information to the shoot, and changes in patterns of expression might induce changes in plant development (Bernstein *et al.* 2013).

### 3.7 Root, Leaf and Stem Fresh and Dry weight

Root fresh and dry weights did not show any statistical difference due to salinity (Table 8). However, significant variations ($p<0.05$) were observed in leaf and stem fresh and dry weights due to salt treatments. The maximum leaf fresh (3.87 g) and dry (1.93 g) weights were recorded at 1.3 dS/m, while the minimum leaf fresh (0.63 g) and dry (0.23 g) weights were recorded at 3.9 dS/m salt concentration.

The maximum stem fresh (7.24 g) and dry (3.62 g) weights were observed at 0 dS/m, while the minimum values of 3.57 g and 1.82 g were recorded at 3.9 dS/m. These values decreased by 40% and 33%, respectively, as the salt concentration increased from 0 to 3.9 dS/m (Table 8).

The results indicate that high salinity may inhibit growth due to slowing down water uptake by the plant, which might be the reason for the decrease (Werner and Finkelstein, 1995). Salinity can rapidly inhibit stem growth and hence the capacity of water uptake and essential mineral nutrition from the soil (Neumann, 1997).

**Table 8.** Effect of salinity level solutions on fresh weight (shoot and root) of avocado seedlings rootstock (Guatemalan race)

| Treatment Salinity (ds/m) | Parameter | | | | | |
|---|---|---|---|---|---|---|
| | Root fresh weight (g plant$^{-1}$) | Root Dry weight (g plant$^{-1}$) | Leave fresh weight (g plant$^{-1}$) | Leaf dry weight (g plant$^{-1}$) | Stem fresh weight (g plant$^{-1}$) | Stem dry weight (g plant$^{-1}$) |
| Control | 5.48 | 2.76 | 2.86$^{b}$ | 1.46$^{b}$ | 7.24$^{a}$ | 3.62$^{a}$ |
| 1.3 | 4.44 | 2.57 | 3.87$^{a}$ | 1.93$^{a}$ | 6.69$^{a}$ | 3.33$^{a}b$ |
| 2.6 | 4.15 | 1.93 | 0.67$^{c}$ | 0.29$^{c}$ | 3.92$^{b}$ | 1.92$^{b}$ |
| 3.9 | 3.74 | 1.75 | 0.63$^{c}$ | 0.23$^{c}$ | 3.57$^{b}$ | 1.82$^{b}$ |
| LSD ($p\leq0.05$) | Ns | NS | 0.21 | 0.55 | 2.06 | 1.541 |
| CV (%) | 19.18 | 21.98 | 13.80 | 23.56 | 23.08 | 22.21 |
| P-value | 0.1826 | 0.09 | 0.0001 | 0.001 | 0.022 | 0.009 |

Means with different letter within the same column are significantly different at ($p<0.05$)

Comparable results are also reported by Dolo (2018), in which he stated that salt stress causes a considerable decrease in leaf fresh and dry weights, as well as roots and stems dry weights. Similarly, Musyimi *et al.* (2007) reported that growth at high salinity concentration resulted in large reductions in fresh and dry weight production of both shoot and root. The reduction in leaf fresh weight was attributed to lower leaf number and development of smaller leaves with increased salinity of the growth medium. Bonomelli *et al.* (2018) also observed that the fresh weight of the aerial part (leaves and stems), roots, and the entire plant in the treatments without the stress (leaf fresh weight and stem fresh weight) were always significantly higher than treatments with saline irrigation.

### 3.8 Leaf Area, Total Fresh weight and Total dry weight

Leaf area, total fresh weight, and total dry weight were significantly ($p<0.05$) affected by salt stress. The highest leaf area (874.29 $cm^2$) was recorded at 0 dS/m, and the minimum leaf area (159.19 $cm^2$) was recorded at 3.9 dS/m (Table 9). It was observed that leaf area decreased with increasing salinity levels, with a decrease of about 81.8% as the salt concentration increased from 0 to 3.9 dS/m.

The decrease in leaf area with increasing salinity levels (Aydinsakir *et al*., 2015; Hasanuzzaman *et al*., 2013) can be attributed to the fact that high salinity hampers cell elongation in the active growing tissue, subsequently reducing the leaf area and dry matter assimilation in the plant.

Similarly, the highest total fresh weight (15.59 g) and dry weight (7.85 g) were recorded at the control (0 dS/m), while the minimum total leaf fresh weight (7.63 g) and dry weight (3.63 g) were recorded at the maximum salt stress level (3.9 dS/m). Concurrently, Bonomelli *et al.* (2018) also reported that cumulative leaf area, fresh weight, and dry weight increase as the salt stress level decreases.

**Table 9.** Effect of salinity level solutions on total leaf area, fresh and dry weight of avocado seedlings rootstock (Guatemalan race)

| Treatments salinity level (ds/m) | Leaf area ($cm^2$) | Total fresh weight (g $plant^{-1}$) | Total dry weight (g $plant^{-1}$) |
|---|---|---|---|
| Control | $874.29^a$ | $15.59^a$ | $7.85^a$ |
| 1.3 | $608.70^b$ | $15.08^a$ | $7.83^a$ |
| 2.6 | $214.82^c$ | $8.02^b$ | $3.83^b$ |
| 3.9 | $159.19^c$ | $7.63^b$ | $3.69^b$ |
| LSD ($p \leq 0.05$) | 185.3 | 4.2 | 4.02 |
| CV (%) | 21.2 | 19.3 | 17.9 |
| P value | 0.013 | 0.03 | 0.01 |

Means with different letter with in the same column are significantly different at ($p \leq 0.05$)

## 3.9 Stomata number, length and width

Stomata number, stomata length, and stomata width were significantly ($p<0.05$) affected by salt stress levels. The maximum stomata number (22/$mm^2$) was recorded at 0 dS/m, while the maximum stomata length (0.35 mm) and width (0.32 mm) were recorded at 1.3 dS/m, which were statistically on par with 0 dS/m.

As the salt concentration increased from 0 to 3.9 dS/m, most of the stomata parameters, including stomata number, length, and width, decreased by 25%, 23.6%, and 13.3% respectively (Table 10). Similar results were also reported by Aydinsakir *et al.* (2015), who found that stomata number decreased with increasing salinity levels.

Comparable results were also reported by Xue *et al.* (2021), who found that water and salt stress reduced stomatal length (SL), width, perimeter, and area (amax), as well as stomatal density (SD). The same trend was also reported on tomato cultivars by Guo *et al.* (2018), where salt stress substantially decreased the stomatal density, stomatal width, stomatal area, and stomatal area index.

**Table 10.** Physiological Response of seedling avocado rootstocks to different salinity level

| Treatment Salinity level (ds/m) | Stomata number (per $mm^2$) | Stomata length ($\mu$m) | Stomata Width ($\mu$m) |
|---|---|---|---|
| 0 | $22.0^a$ | $0.34^a$ | $0.28^a b$ |
| 1.3 | $18.6^b$ | $0.35^a$ | $0.32^a$ |
| 2.6 | $15.3^c$ | $0.24^b$ | $0.22^b$ |
| 3.9 | $13.6^c$ | $0.21^c$ | $0.21^b$ |
| LSD ($p \leq 0.05$) | 2.1 | 0.08 | 4.13 |
| CV (%) | 6.46 | 15.73 | 8.61 |
| P value | 0.0001 | 0.008 | 0.002 |

Means with different letter within the same column are significantly different at ($p \leq 0.05$)

### 3.10 Photosynthesis, Transpiration, Stomata conductance and Instantaneous water use efficiency (IWI)

Physiological parameters such as photosynthetic rate, transpiration, gas exchange (gs), and water use efficiency were significantly (P<0.05) influenced by salt treatments (Table 11). The transpiration rate decreased by 60% as the salt concentration increased from 0 to 3.9 dS/m, a similar result reported by Aydinsakir *et al.* (2015).

Likewise, the photosynthetic rate decreased by 51.8% as salinity levels increased from 0 to 3.9 dS/m. Hnilickova *et al.* (2021) also reported that as the concentration of $NaCl$ reached 100 mM, they found a decrease in stomatal conductance simultaneously with an increase in $CO_2$ assimilation (A). The photosynthesis rate can drop due to stomatal closure (gas) and/or other non-stomatal limitations, like the disturbance of the photosynthetic electron chain and inhibition of Calvin cycle enzymes (Chaves *et al.*, 2009). A drop in gas exchange can prevent excess water loss by transpiration, whereas proper regulation of the photosynthetic process can minimize the generation of ROS in PS2 and in the reducing side of the PS1 (Asada, 1999).

Similarly, Musyimi *et al.* (2007) reported that the decline in net photosynthesis with increasing salinity is associated with a similar reduction in gs in salt-treated plants. Reduction in photosynthesis is directly related to stomatal conductance, though non-stomatal factors are also associated with lower photosynthetic capacity in salt-treated plants (Ashraf, 2002; Netondo *et al.*, 2004). Schaffer and Whiley (2003) have indicated that stomatal conductance is a more reliable early indicator of salt stress in avocado than measurements of leaf water content, leaf water potential, or other growth variables.

Redondo-Gómez *et al.* (2007) found that the net photosynthetic rate (A) declined significantly with increasing external salinity after 6 days of treatment. Guo *et al.* (2018) reported that $NaCl$ stress resulted in marginal declines in the net photosynthetic rates (Pn), stomatal conductance (gs), and transpiration rates (Tr) of avocado cultivars they used in their study. Similar observations were also reported in mature avocado trees irrigated with an EC = 1.5 dS/m salinity level (Acosta-Rangel *et al.*, 2019), where the avocado leaves visibly damaged by the salinity (named as partially burned leaves) experienced photoinhibition and reduction of photosynthetic rate and water-use efficiency, suggesting that the poor performance in carbon assimilation contributed to reductions in yield and increases in mortality.

**Table 11.** Response of avocado seedlings rootstock (Guatemalan race) to different physiological growth parameter under different salinity level

| Avocado (GT) | Parameter | | | |
|---|---|---|---|---|
| Treatment salinity level (ds/m) | Photosynthesis rate ($\mu$m) | Transpiration rate (E: mmol/mol) | Stomata conductance (gs: mmol) | IWUE |
| Control | $5.40^{a}$ | $3.05^{a}$ | 116.6 | $2.84^{a}$ |
| 1.3 | $4.63^{a}b$ | $2.72^{a}b$ | 110.6 | $2.75^{a}$ |
| 2.6 | $3.96^{a}b$ | $2.26^{a}b$ | 71.6 | $2.35^{a}b$ |
| 3.9 | $2.60^{b}$ | $1.29^{b}$ | 57.6 | $1.48^{b}$ |
| LSD ($p \leq 0.05$) | 2.2 | 1.2 | 61 | 1.19 |
| CV (%) | 23.8 | 27.7 | 30.5 | 23.2 |
| P value | 0.04 | 0.04 | 0.07 | 0.05 |

Means with different letters within the same column are significantly different at (p<0.05)

## 4 Conclusion and Recommendation

Ethiopia's agroecological conditions favor the cultivation of different fruit crops of tropical, subtropical, and temperate zone origins. However, these crops are exposed to various biotic and abiotic stress conditions. Among abiotic stresses, salinity is one of the environmental stresses that adversely affects growth, physiology, and yielding potential. The avocado plant is very sensitive to such stress prevalent in the subtropics. However, there are limited studies that have focused on the performance of avocado seedlings under such stress conditions.

The study indicated that salt stress impairs the morphological and physiological performance of grafted avocado seedlings. As the salinity level increases, both rootstock and scion diameter, as well as the grafting success percentage, were substantially decreased compared to the salt-free treatment. Except for the number of suckers on the root part, which shows an increasing trend, as the salt concentration increased from 0 to 3.9 dS/m, most of the vegetative growth parameters decreased with increasing salt concentration. These parameters include the number of buds, leaf number, leaf length, stem diameter, plant height, scion length, and scion and rootstock diameter.

Growth at high salinity resulted in large reductions in the fresh and dry weight production of both the shoot and root parts of avocado seedlings. The observed fresh and dry weight of the aerial part (leaves and stems) and underground root parts were significantly higher in the treatments without the stress compared to the treatments with saline conditions.

Avocado seedlings treated with higher salinity levels significantly reduced stomata (number, length, and width), stomatal conductance, and photosynthetic rate. The control treatment without salt growing conditions significantly increased all growth and physiological parameters compared to the salt stress conditions.

The irrigation water with an EC of <1.3 dS/m positively affected both vegetative growth and physiological performance, while salinity levels >1.3 dS/m negatively affected most of the morpho-physiological performance of avocado seedlings. Therefore, considerable attention should be given to the irrigation water quality for avocado seedling production at nursery sites to ensure the development of vigorous seedlings.

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