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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

Salinity represents a primary environmental constraint that negatively impacts the morpho-physiological performance of avocado. As a result, this study was conducted to evaluate the response of avocado seedlings to different levels of salt stress. The experimental design was structured as a Randomized Complete Design (RCD) featuring four levels of salt concentration (0, 1.3 dS/m, 2.6 dS/m, and 3.9 dS/m) applied to grafted avocado seedlings, with three replications for each treatment. The avocado seedlings were cultivated from Ettinger avocado scions grafted onto Guatemalan race rootstock. The findings reveal that the majority of the morphological growth parameters, including rootstock diameter, sucker development, and root length, were significantly ($p<0.01$) influenced by the level of salt stress. In contrast, tap root diameter and lateral root length did not show significant ($p>0.05$) changes due to salt treatment. Notably, with the increase in salt concentrations from 0 to 3.9 dS/m, both the rootstock and scion diameters significantly decreased, along with the number of suckers developed on the rootstock. Additionally, significant ($p<0.01$) differences were noted in leaf length, bud count, leaf fresh weight and dry weight, leaf area, shoot height, taproot length, and lateral root length, all of which were significantly reduced as salt concentrations increased. Furthermore, photosynthesis, transpiration, stomatal conductance, and instantaneous water use efficiency were significantly impacted by salt concentration, with the highest values recorded 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

The avocado (*Persea americana* Mill.) is indigenous to Mexico and Central America, classified under the Lauraceae family, and ranks among the most economically significant tree crops globally (Silva and Ledesma, 2014). It offers numerous benefits, especially regarding its nutritional profile, being abundant in monounsaturated fatty acids (MUFA), dietary fiber, essential nutrients, and phytochemicals (Fulgoni *et al*., 2013). Additionally, it serves as shade trees, windbreaks, and ornamental plants (Albertin and Nair, 2004). Over the past century, it has been introduced to more than 50 countries worldwide, particularly in sub-tropical and tropical areas, with Ethiopia recognized as one of the leading avocado producers in tropical Africa.

Despite Ethiopia's long history of introduction and suitable agro-ecological conditions (Wayessa and

Berhanu, 2010), its distribution remains limited to a few areas (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 represent the primary constraints in harnessing the potential of horticultural crops globally (Celis *et al.*, 2018). Among these stresses, salinity, temperature, nutrition, light, oxygen availability, and metal ion concentration are the most critical factors. Salt stress is a significant adverse environmental factor that affects physiological processes by modifying the osmotic conditions within and surrounding the plant's environment (Jouyban, 2012). Elevated salt concentrations in the external solution of plant cells can lead to various consequences, which can be categorized into three distinct types: osmotic drought, toxicity resulting from excessive 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.

Saline irrigation water tolerance is an increasingly critical trait for avocado rootstocks, given the crop's high sensitivity to salt (Grieve *et al.*, 2012). Plants employ various mechanisms to withstand saline conditions, broadly categorized (Roy *et al.*, 2014) into three main types. These mechanisms are governed by long-range signals that inhibit shoot growth, commencing prior to significant Na+ accumulation in the shoots. The initial mechanism involves ion exclusion, wherein roots restrict the buildup of harmful Na+ and Cl– concentrations in the leaves. The subsequent mechanism is tissue tolerance, which is defined by increased salt concentrations in the leaves that are compartmentalized at both the cellular and intracellular levels, especially within vacuoles (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 CO2 assimilation, chlorophyll concentration, and leaf necrosis.

Mild water and salt stress improved intrinsic water use efficiency (iWUE = A/gs) by (i) decreasing gs through increased hydraulic resistance and osmotic adjustment, and (ii) decreasing A due to increased stomatal limitations rather than decreased photosynthetic capacity, according to Liao *et al.* (2022).

Furthermore, overall plant growth, net photosynthetic rate (PN), stomatal conductance (gs), transpiration rate (E), water use efficiency (WUE), and chlorophyll (chl) content are all significantly impacted by salinity, as shown by Musyimi *et al.* (2007). Avocado development and gas exchange are hampered by high salt concentrations.

Celis *et al.* (2018) also observed significant leaf burn in rootstocks grown in salt-treated soils. The occurrence of "tip-burn" on leaves in the autumn indicates excessive chloride uptake, leading to premature leaf drop during the winter months. Subsequent leaf regeneration can compromise flowering and fruit set, contributing to persistently low yields in certain orchards.

Rootstocks exhibit variability in their capacity to absorb and translocate chloride and sodium. Guatemalan rootstocks possess an intermediate level of salinity tolerance. However, comprehensive research on the comparative salinity tolerance of various avocado rootstock races remains limited. Therefore, evaluating the performance of avocado seedlings at an early developmental stage under varying salinity concentrations is crucial for determining the appropriate quality of irrigation water and soil media utilized in nurseries. In Ethiopia, data regarding the response of avocado seedlings to saline conditions are scarce. Consequently, this study investigates the morpho-physiological responses of grafted avocado seedlings to different salinity levels in the Sidama Region, Southern Ethiopia.

2 Materials and Methods

2.1 Description of the Study Area

The research was carried out between September 2021 and October 2022 at a site in the Dara district of Tafari-Kella, situated in the Sidama National Regional State (see Fig 1). This location is approximately 350 kilometers south of Addis Ababa, at an elevation of 1850 meters above sea level, positioned at a latitude of 6°30’0"N and a longitude of 38°24’0"E. The area receives an average annual rainfall of 1700 millimeters and has a typical temperature of 27 degrees Celsius. The prevalent agricultural system is characterized by coffee fruit cultivation, with avocado being the primary fruit crop.

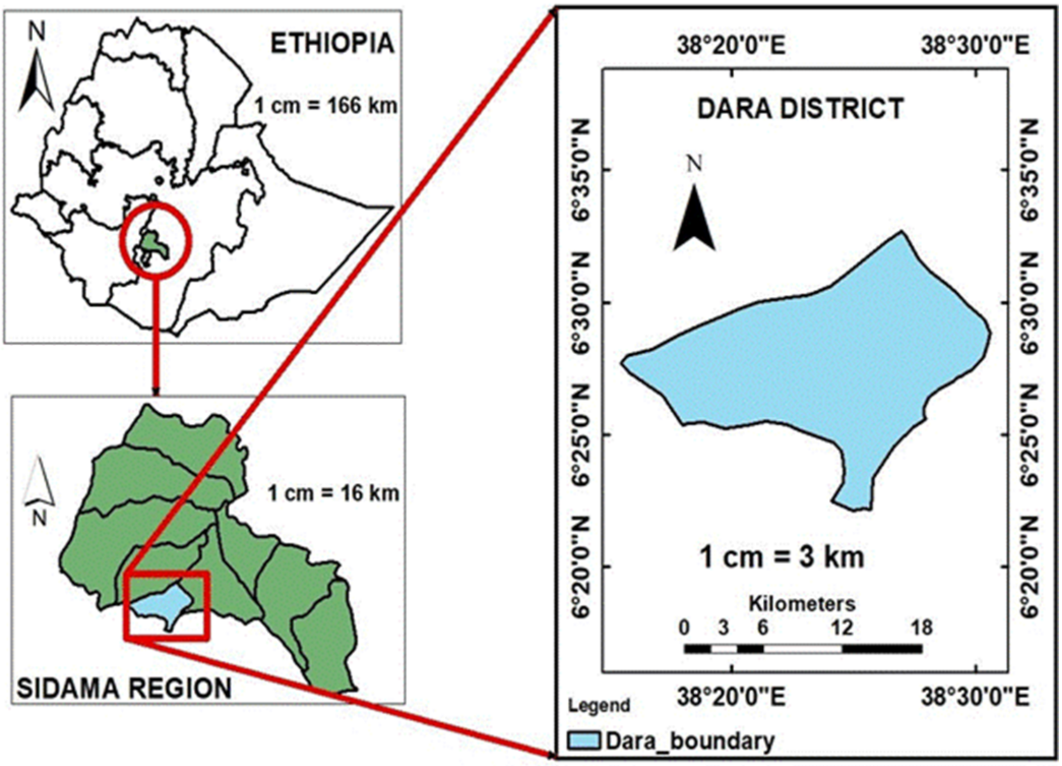

Figure 1. Map of the study area (Dara district Tafari- Kella kebele Sidama National Reginal State, Ethiopia)

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 maximum and minimum air temperatures for the shaded house were documented on five chosen days throughout the experimental period, utilizing the temperature and relative humidity data logger known as "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°C and 32.4°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 carried out in a plastic shade using pots measuring 16 x 20 cm, which were filled with a composite soil made up of a 3:2:1 ratio of topsoil, compost, and sand, respectively. The study employed a Completely Randomised Design (CRD) with three replications. Each experimental unit consisted of four pots for each 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 Characteristics of the Experimental Soil

A soil sample was taken from the mixed soil that was ready for planting in order to ascertain the physicochemical characteristics of the soil utilized for the experiment. Prior to laboratory analysis, the composite sample was dried and pulverized until it could fit through a 0.2 mm sieve. Following routine protocols at the Hawassa soil laboratory, the samples were examined for study-relevant parameters such pH, texture class, organic carbon, total nitrogen, accessible phosphorus, CEC, FC, and EC (Table 1).

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. The root system morphology was assessed by measuring root length and tap root diameter. Plant biomass was determined by quantifying leaf fresh weight, stem fresh weight, leaf dry weight, and root dry weight (all expressed in grams), as well as leaf area (cm2). Total fresh and dry weights were also recorded. All data were collected according to established methodologies, as detailed by Ndoro *et al*. (2018).

2.5.3 Stomata anatomy

One hundred twenty days after the therapy was administered, stomata anatomy was measured using the method suggested by Xu and Zhou (2008). An Automated Upright Leica Microscope DM5000 B with a 40x magnification lens fixed with a digital Leica DFC425/DFC425C image processing camera was used to investigate the stomata print. Stomata number (per mm^2), stomata cell number (per mm^2), stomata opening (μm), and stomata length (μm) were measured for every sample.

2.5.4 Gas exchange and physiological parameters

The third young and completely developed leaves of three randomly chosen seedlings were used to measure the following parameters: photosynthesis (A, μmol m^{-2} s^{-1}), transpiration rate (E), stomatal conductance (gs), and water usage efficiency. These measurements were made using a CIRAS-3 portable photosynthetic system (CIRAS-3 PP System Inc., Lincoln, NE, USA). The measurements were made 45 days after the completely grown, intact leaves were subjected to the actual moisture treatment.

A leaf surface area of 6.25 cm^2, ambient CO_2 concentration of 386 μmol mol^{-1}, leaf chamber mass flow rate of 251 μmol s^{-1}, atmospheric pressure of 840 bar, and manually fixed photosynthetic active radiation (PAR) at 600 μmol m^{-2} s^{-1} were all maintained during the measurements, which took place between 9:30 AM and 12:30 PM.

2.6 Statistical Analysis

ANOVA was performed on all morphological and physiological data in accordance with the General Linear Model (GLM) standard approach (Gomez and Gomez, 1984). SAS version 9.0 was used to conduct statistical analyses (SAS, 2008). Fisher's Least Significant Difference (LSD) test was used to differentiate treatment means at the 0.05 probability level.

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

Salt stress levels had a statistically significant ($p<0.05$) impact on rootstock diameter and the number of suckers that grew on the rootstock, according to analysis of variance. Scion diameter, however, was not significantly impacted.

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. Nevertheless, Table 3 shows that there was no discernible difference in scion diameter between the various salinity levels.

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. According to earlier research (Mandal *et al.*, 2012; Pampanna and Sulikeri, 1995), the maximum number of leaves may be the result of photosynthetic accumulation in recently grafted plants, which in turn boosted the number of nodes and the leaf primordia's ability to absorb moisture and nutrients.

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

Salinity levels significantly impacted stem diameter, plant height, scion diameter, and grafted success percentage on the rootstock ($p < 0.05$). Plant height exhibited a significant negative correlation with increasing salinity concentration.

The 1.3 dS/m salt concentration produced the tallest plants (22.6 cm), followed by the control (0 dS/m) and 2.6 dS/m salinities (21.2 cm and 20.33 cm, respectively) (Table 5). The lowest plant height (18.33 cm) was recorded from 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). Avocado plants exposed to higher soil salinity levels also demonstrated a significant decrease in plant height, as confirmed by Berkessa (2020) and Bernstein *et al*. (2004). This could be due to a variety of physiological reactions, including altered ion balance, altered water status (water potential) both inside and outside the plant cell, mineral nutrition processes, and other aberrant metabolic processes.

Salinity-induced reductions in plant height are correlated with the accumulation of reactive oxygen species (ROS), which, at elevated concentrations, can be detrimental due to their high reactivity and potential to induce destructive processes and cellular damage (Kesawat *et al*., 2023). Prior research has established the role of rootstocks in modulating salt tolerance in grafted fruit trees, including avocado (Cooper, 1951; Haas, 1950). Concordantly, scion leaf production in avocado is inversely related to salinity levels (Oster and Arpaia, 1992), with the magnitude of growth reduction under stress varying among different rootstocks.

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 plants that were treated with 3.9 dS/m salt had the most suckers (1.9 per plant), while the plants treated with 0 dS/m salt had the fewest suckers. 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.

The current results are consistent with those of Castro *et al.* (2009), Bhatia and Kumar (2011), and Rana and Bhatia (2004), who noted that rootstock diameter increased in response to salt stress. Conversely, Bonomelli *et al.* (2018) reported stimulated vegetative growth in avocado trees under low salinity conditions in Mexican plants, attributing this to enhanced cellular growth and increased cell number via osmoregulation. This observation is further supported by Penella *et al.* (2016), who demonstrated that salt stress significantly inhibited plant growth, resulting in a 40.6% reduction in leaf dry weight compared to 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}	$20.80^{a}b$
1.3	1.16^{b}	2.16^{a}	22.00^{a}
2.6	1.86^{a}	$1.60^{a}b$	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 control treatment had the greatest number of roots per plant (22.4), while the lowest number (9.9) was found at 3.9 dS/m. Additionally, it was noted that when saline levels rose, fewer roots were found (Table 7). Aydinsakir *et al.* (2015) also reported a similar outcome.

While root length, tap root diameter, and lateral root diameter did not exhibit statistically significant differences ($p>0.05$), root length showed an 18.9% increase, rising from 3.74 to 5.48 cm, with an increase in salt concentration from 0 to 3.9 dS/m. Conversely, tap root and lateral root diameters decreased from 4.9 to 3.9 mm and from 1.09 to 0.65 mm, respectively, as 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 ($p \leq 0.05$)

Since roots are in close touch with the soil and take up water from it, while shoots provide water to the plant's aerial parts, it is well recognized that plant height and root length are the most significant salinity characteristics (Jamil and Rha, 2004). Usually, the main reaction to stress is the suppression of shoot and root development. Salinity has an impact on the growth, morphology, anatomy, and physiology of roots. Plant development may be affected by modifications in the roots' intake of water and ions, the synthesis of hormone signals that inform the shoot, and shifts in expression patterns (Bernstein *et al.* 2013).

3.7 Root, Leaf and Stem Fresh and Dry weight

Root fresh and dry weights were not substantially affected by salinity (Table 8). However, the fresh and dry weights of the leaves and stems varied significantly (p<0.05) as a result of the salt treatments.

Maximum leaf fresh (3.87 g) and dry (1.93 g) weights were observed at a salinity level of 1.3 dS/m, while minimum leaf fresh (0.63 g) and dry (0.23 g) weights were recorded at 3.9 dS/m.Stem fresh (7.24 g) and dry (3.62 g) weights were highest at 0 dS/m. The lowest stem fresh (3.57 g) and dry (1.82 g) weights were recorded at 3.9 dS/m, representing a decrease of 40% and 33%, respectively, as salinity increased from 0 to 3.9 dS/m (Table 8).

The findings suggest that excessive salinity may hinder growth by delaying the plant's absorption of water, which could be the cause of the decline (Werner and Finkelstein, 1995). According to Neumann (1997), salinity can quickly impede stem growth and, consequently, the ability to absorb water and vital mineral nutrients from the soil.

Similar results were reported by Dolo (2018), who found that salt stress dramatically reduces the fresh and dry weights of leaves as well as the dry weights of roots and stems. Similarly, Musyimi *et al.* (2007) found that high saline concentrations significantly decreased the fresh and dry weight production of both shoots and roots. The reduction in leaf number and the formation of smaller leaves as a result of the growth medium's increased salinity were cited as the causes of the drop in leaf fresh weight. The fresh weight of the roots, aerial parts (leaves and stems), and the total plant in control treatments (leaf fresh weight and stem fresh weight) were consistently and considerably higher than those that received saline irrigation, according to Bonomelli *et al.* (2018).

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 ($p \leq 0.05$)	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)

3.8 Leaf Area, Total Fresh weight and Total dry weight

Salt stress had a substantial impact on leaf area, total fresh weight, and total dry weight (p < 0.05). At a salinity level of 0 dS/m, the largest leaf area measured was 874.29 cm2, and at 3.9 dS/m, the smallest leaf area measured was 159.19 cm² (Table 9). As salinity levels increased, leaf area showed a decrease of approximately 81.8%, dropping from 0 to 3.9

dS/m.

High salinity inhibits cell elongation in actively growing tissues, which in turn affects leaf area and dry matter assimilation in the plant. This is the reason for the decrease in leaf area that occurs when salinity levels rise (Aydinsakir *et al*., 2015; Hasanuzzaman *et al*., 2013).

In parallel, the highest total fresh weight was observed at 15.59 g and the highest dry weight at 7.85 g at the control level (0 dS/m). Conversely, the lowest total fresh weight (7.63 g) and dry weight (3.63 g) were noted at the maximum salt stress level of 3.9 dS/m. Similarly, Bonomelli *et al.* (2018) reported that cumulative leaf area, fresh weight, and dry weight increase as the level of salt stress 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

Levels of salt stress had a substantial ($p<0.05$) impact on stomata number, length, and width. While the maximum stomata length (0.35 mm) and width (0.32 mm) were observed at 1.3 dS/m, which were statistically equivalent to 0 dS/m, the greatest stomata number (22/mm2) was reported at 0 dS/m.

The majority of the stomata metrics, such as stomata number, length, and width, reduced by 25%, 23.6%, and 13.3%, respectively, when the salt concentration rose from 0 to 3.9 dS/m (Table 10). Aydinsakir *et al.* (2015) also reported similar findings, indicating that the number of stomata dropped as saline levels increased.

Water and salt stress decreased stomatal length (SL), width, perimeter, and area (amax), as well as stomatal density (SD), according to similar findings reported by Xue *et al.* (2021). Guo *et al.* (2018) found that salt stress significantly reduced the stomatal density, stomatal width, stomatal area, and stomatal area index in tomato cultivars.

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

Treatment Salinity level (ds/m)	Stomata number (per mm^2)	Stomata length (μm)	Stomata Width (μm)
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)

Salt treatments had a substantial (P<0.05) impact on physiological measures like photosynthetic rate, transpiration, gas exchange (gs), and water use efficiency (Table 11). As the salt level rose from 0 to 3.9 dS/m, the transpiration rate decreased by 60%; Aydinsakir *et al.* (2015) found a similar finding.

Similarly, when salt levels rose from 0 to 3.9 dS/m, the photosynthetic rate dropped by 51.8%. Additionally, Hnilickova *et al.* (2021) revealed that stomatal conductance decreased concurrently with an increase in CO2 absorption when the concentration of NaCl reached 100 mM (A). Stomatal closure (gas) and/or other non-stomatal constraints, such as disruption of the photosynthetic electron chain and inhibition of Calvin cycle enzymes, can cause the rate of photosynthesis to decrease (Chaves *et al.*, 2009). While proper control of the photosynthetic process can reduce the generation of reactive oxygen species (ROS) in photosystem II (PS2) and the reducing side of photosystem I (PS1), a decrease in gas exchange can assist prevent excessive water loss through transpiration (Asada, 1999).

Similarly, research by Musyimi *et al.* (2007) has demonstrated that in plants treated with salt, a decrease in stomatal conductance (gs) is correlated with a decrease in net photosynthesis brought on by rising salinity. Although stomatal conductance is the main cause of the decrease in photosynthesis in salt-treated plants, non-stomatal variables also have a role (Ashraf, 2002; Netondo *et al.*, 2004). Stomatal conductance is a more accurate early indication of salt stress in avocados than studies of leaf water content, leaf water potential, or other growth indicators, according to Schaffer and Whiley (2003).

According to Redondo-Gómez *et al.* (2007), after six days of treatment, the net photosynthetic rate (A) dramatically decreased as external salinity increased. According to Guo *et al.* (2018), the avocado cultivars they utilized in their study showed slight decreases in net photosynthetic rates (Pn), stomatal conductance (gs), and transpiration rates (Tr) under NaCl stress. Similar findings were also documented in mature avocado trees that were irrigated with an EC = 1.5 dS/m salinity level (Acosta-Rangel *et al.*, 2019). The avocado leaves that were clearly harmed by the salinity—referred to as partially burned leaves—experienced photoinhibition, a decrease in photosynthetic rate, and a decrease in water-use efficiency. These findings suggest that the poor performance in carbon assimilation contributed to decreased yield and increased 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 (μm)	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^ab	2.72^ab	110.6	2.75^a
2.6	3.96^ab	2.26^ab	71.6	2.35^ab
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. Nevertheless, a variety of biotic and abiotic stress conditions affect these crops. Salinity is one of the environmental factors that negatively impacts development, physiology, and yielding potential among abiotic stresses. The avocado plant is particularly vulnerable to this kind of stress that is common in subtropical regions. Nevertheless, the performance of avocado seedlings under such stress conditions has not been extensively studied.

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. The majority of the vegetative development metrics declined as the salt concentration rose from 0 to 3.9 dS/m, with the exception of the number of suckers on the root section, which exhibits an increasing tendency. The number of buds, number of leaves, length of leaves, stem diameter, plant height, scion length, and diameter of the scion and rootstock are some of these factors.

Both the shoot and root portions of avocado seedlings produced much less fresh and dry weight when grown in high saline environments. In comparison to the treatments with saline circumstances, the aerial component (leaves and stems) and underground root parts' observed fresh and dry weights were significantly higher in the stress-free treatments.

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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