Root Structure and Function of Grapevine Rootstocks (*Vitis*) in Response io Salinity

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Research Article

Keywords: Grapevine, rootstock, roots, salinity, plant biomass.

Posted Date: August 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-805129/v1

Abstract

Purpose

Accessing freshwater resources becomes more complex in arid and semi-arid areas due to increased demands and declining water quality. Alternative water sources for agriculture such as saline and recycled water are currently being used. A better understanding of roots' response to irrigation with saline water is crucial for future agriculture in arid and semi-arid areas.

Methods

Three grapevine (*Vitis*) rootstocks were examined, and their roots' responses to salinity were studied. The rootstocks were planted in pots filled with sand and were grown in a commercial net house subjected to two salinity treatments: 10 m*M* and 30 m*M* NaCl (EC = 2 and 4 ds m^{-1}, respectively). We measured root morphologic and anatomic properties at the end of the experiment.

Results

The specific root area increased in response to salinity due to reduced root tissue density. In addition, a reduction in the average root diameter also affected the specific root area by increasing the surface area to volume ratio. Plant biomass was allocated primarily to the shoot in all three rootstocks, reducing the root to shoot ratio. At the same time, the bottom part of the root zone was more affected by salinity. SO4 showed improved chloride and sodium exclusion, concomitant with a significant increase in its narrow roots' contribution to the surface area.

Conclusion

Narrow roots play a more prominent role in the acquisition of water and nutrients as salinity increases. Furthermore, a decrease in root tissue density and average diameter may contribute to salt exclusion from the roots.

Introduction

Freshwater scarcity can arise from climate changes, competition with domestic and industrial sectors, pollution and salinization of underground water, world population growth, and increased living standards. As agriculture utilizes more than 70% of the world's freshwater resources (FAO 2020), finding diversified water sources is one solution to meet the growing demand. Underground water is the source of one-third of globally freshwater use, supplying an estimated 42% of the water used for agriculture (Taylor et al. 2012). However, due to the dissolving of salts in the soil, mainly in arid and semi-arid regions, this underground water tends to have higher salinity levels than aboveground water (Connor et al. 2017). Another sustainable water source, in addition to belowground water, is recycled water (Dolnicar and Schäfer 2009). However, the use of recycled municipal wastewater poses some problems, salinity among them (Higgins et al. 2002). Belowground water and recycled

water could be exploited due to their relatively low cost. But their introduction in agriculture requires ad-hoc applications and salt-tolerant crops, limiting the potential use due to salt accumulation.

Grapevine (*Vitis vinifera*) is an important crop worldwide, including in arid and semi-arid areas (Cifre et al. 2005). Grapevines are considered moderately sensitive to salt (Hillel 2000). Under saline conditions, grapevines were shown to decrease photosynthesis, leaf area, and total dry mass (Fisarakis et al. 2001). In addition, decreases in leaf sucrose and starch levels were shown (Downton 1977) and, eventually, yield reduction (Shani and Ben Gal 2005). Sodium chloride (NaCl) is the most common salt in the world and for most crops, sodium (Na^+) is the more toxic ion. Therefore, the majority of the published data deals with Na^+ excluding mechanisms. Grapevines, on the other hand, are good Na^+ excluders, thus preventing it from reaching toxic levels, making chloride (Cl^-) more toxic for them. Plants can exclude salt from their leaves by root exclusion or by allocating unbeneficial ions to woody tissues using specific ion transporters (Munns and Tester 2008).

The choice of a suitable rootstock is a widely accepted strategy to contend with the effects of biotic and abiotic hazards, such as salinity (Colla et al. 2010). Grafting has a significant impact on the whole plant's performance. However, the outcome of grafting depends on the interaction between the genotypes involved. Each cultivar has its own genetic, morphological, and physiological properties (Martínez-Ballesta et al. 2010) and, consequently, can provide various levels of trait modulations when grafted onto a different genotype. In addition, a rootstock can determine water and nutrient uptake, plant growth, hormone levels and storage reserves (Goldschmidt 2014; Nawaz et al. 2016 Schwarz et al. 2010). In grapevines, carbohydrates play essential roles in berry development among other plants, and a reduction in carbohydrates negatively affects berry quality (Rossouw et al. 2017). Consequently, the efficacy of the rootstock in producing roots is an important trait that can lessen the effects of carbohydrate reduction caused by salinity. Moreover, root systems can influence the buildup of Na^+, Cl^-, and K^+ (potassium) in grapevines by differentially regulating their uptake, which is expressed by changes in the ion compositions in the leaves and berries (Downton 1997). We chose to work with three commercially available rootstocks broadly used in semi-arid areas and vary in salt tolerance: Paulsen 1103, Richter-110 (R-110), and Sélection Oppenheim 4 (SO4). Paulsen was found to be the most salinity tolerant of the three in terms of scion vigor, while SO4 was the least tolerant (Corso and Bonghi 2014).

Recently, some models and experiments have shown that changes in root system architecture can improve the survival rate of plants and alleviate the adverse effects caused by abiotic stresses, such as drought (Veerappa et al. 2019), phosphate deficiency (Heppell et al. 2015), waterlogging (Ye et al. 2018), and salinity (Koevoets et al. 2016; Perelman et al. 2020; Schröder et al. 2014), as well as by indirect factors, such as carbon limitation. Furthermore, Lynch (2007) suggested that roots must be more metabolically efficient due to plants' unfavorable conditions. However, there is currently insufficient knowledge of how roots adapt to salinity stress and maintain enough surface area for water and nutrient acquisition. Therefore, we hypothesized that salinity stress would change the root system architecture and the structure of individual roots. This study aims to understand the mechanism by which grapevines can become more salt-tolerant and integrate the root system and the efficiency of root formation, which may improve the understanding of characteristics contributing to salinity tolerance.

Materials And Methods

Experimental design

The experiment was conducted in a net house at the Ben-Gurion University of the Negev, the Jacob Blaustein Institutes for Desert Research (30°51'16.6"N / 34°47'00.4"E, 473 m above sea level). The experiment was conducted in 2019, between May 16th and August 6th, a total of 82 days. Midday temperatures ranged from 27 to 40.9 ^{o}C, and midday relative humidity ranged from 5% to 47% (based on a meteorological station located 2 km from the net house). One-year-old seedlings were planted in 18-l pots filled with sand (~85% sand, ~10% silt, and ~5% clay). A white plastic lid covered the top of each lysimeter to minimize soil evaporation. The vines were irrigated using a drip irrigation system (1.2 l/h pressure compensating drippers) connected directly to a tank containing the final fertilizer and salt solutions. Plants were pruned before bud bursts, keeping 4 buds on each plant. The shoots were pruned after budding, allowing two main shoots to grow. Flowers were removed to minimize variables. A 16.7% Hoagland solution was used for fertilization to supply 40 ppm of nitrogen (Hoagland and Arnon 1950). A pipe filled with rockwool was connected to the bottom of each pot to improve aeration and leaching in the root system (Ben-Gal and Shani, 2002).

Three commercially used rootstock cultivars were tested in the experiment: Richter-110 (R-110) (*Vitis berlandieri* × *Vitis rupestris*), Selection Oppenheim 4 (SO4) (*Vitis berlandieri* × *Vitis riparia*), and Paulsen-1103 (*Vitis berlandieri* × *Vitis rupestris*) using eight replicates, with 72 plants in total. Five replicates from each cultivar-treatment combination were taken for root analysis. The vines were trellised using cables 1.5 m above the plants, maximizing leaf light exposure while minimizing humidity in the canopy. Plants and treatments were randomly distributed in the greenhouse. Drainage was collected for performing water balance.

Three salinity levels were used: 1) low (control), electrical conductivity (EC) = 0.7 dS m^{-1} (tap water + fertilizer); 2) moderate, EC = 2 dS m^{-1} (tap water + fertilizer + 10 milimolar (m*M*) NaCl); and 3) high, EC = 4 dS m^{-1} (tap water + fertilizer + 30 m*M* NaCl). The plants were excessively irrigated, maintaining a leaching fraction of 33% to prevent water deficiency or NaCl accumulation in the root system. At the end of the experiment, all plants were harvested and measured for biomass, morphological properties, root anatomy, and ion concentrations in the young leaves and fine roots.

Physiological measurements

Total transpiration was measured by collecting and weighing the drainage, then subtracting it from the amount of water supplied to the plants. Evaporation was assumed to be negligible due to the covers on the pots and minimal soil water storage change due to the high leaching fraction. Water use efficiency (WUE) was calculated as the total amount of water transpired divided by the dry mass of the shoot.

Morphological measurements

Dry plant biomass was measured at the end of the experiment. First, plants were separated into roots, stems, and leaves. Starting from the root crown, the root system was separated into the top (above 15 cm) and bottom (below 15 cm). The entire root system was taken out from the pot, cut in the middle (15 cm from the root crown) and washed from sand. The leaves and the stems were dried in an oven at 65 ºC for 48 and 72 h, respectively. Aboveground mass (AGM) was calculated by the sum of the leaves and stems. After the roots were measured

for morphological data, they were washed and dried in the same manner as the stems. The root to shoot ratio was calculated using the dry mass of the roots and the dry mass of the AGM.

Root surface area, root length, root volume, and root average diameter were measured with a scanner EPSON expression 10000 XL (Epson America, Inc. Long Beach, CA, USA). Then images were analyzed with WinRHIZO software (Regent Instruction, Quebec, Canada). Root length density (RLD) was calculated for different root diameters: 0–1 mm, 1–2 mm, 2–3 mm, 3–4 mm, and all roots with a diameter above 4 mm. The RLD was calculated by the ratio of the root length (cm) per soil volume (cm^3). Root tissue density was calculated based on the ratio of root dry mass and root volume (Birouste et al. 2014). Specific root area (SRA) was calculated by the ratio of root area (m^2) to root dry mass (kg; Lõhmus et al. 1989). D-50 and D-90 represent the root diameters responsible for 50% and 90% of the cumulative root system surface area, respectively, and were calculated from the data using a cubic polynomial model. Diameter and density response for each cultivar under 10 m*M* and 30 m*M* of NaCl were calculated by:

$$Response\ relative\ to\ control\ (\%) = \frac{Treatment - Control}{Control} \times 100$$

Root anatomy

Root samples were taken during harvest from six control plants and six plants from the 30 m*M* treatment. Two lateral roots were used from each plant. Samples were kept in 70% ethanol at 4 °C before preparation. Root segments were taken at 15 and 40–50 mm from the tips and underwent dehydration via the protocol described by Hochberg et al. (2013). Cross-sections of 8 mm in thickness were made using a microtome (RM2235, Leica, Nussloch, Germany) and then fixed to glass slides. The samples were de-paraffinized, rehydrated, and stained with aniline blue. ImageJ software was used to analyze the images (Abràmoff et al. 2004), taken by the optical microscope (Zeiss Stemi SV6, Carl Zeiss, Jena, Germany) using a Zeiss high-resolution digital camera.

Soluble ion analysis

Soluble ion analysis was carried out at the end of the experiment. Fine root samples (< 2 mm) were taken from the top and bottom parts of the root system and then uniformly mixed. After drying, as described above, the samples were grounded using a mixed mill 400 (Retsch, Haan, Germany). Cl^- analysis was done by mixing 50 mg of dry sample with 5 ml of double-distilled water, shaken overnight, and filtered using filter paper MN 640 md. All Cl^- analyses were carried out using a model 926 Cl^- analyzer (Sherwood Scientific Ltd., Cambridge, UK). Na^+ and K^+ analysis was done by an inductively coupled plasma–optical emission spectrometr (ICP-OES; Varian 720-ES, Varian Inc, CA, U.S), using 100 mg of dry sample, digested with 0.05 mM of nitric acid following Munns et al. (2010). Ion levels were calculated by mmol to gram of dry mass.

Statistical analysis

All data were tested for homogeneity of variance by Levene's test and for normal distribution by the Shapiro-Wilk test. A data transformation was used in case of violation of assumption, but for simplicity, the original data are presented. A one-way ANOVA was used to examine the effect of treatments on biomass allocation, D-50 and D-90, Cl^- levels, and SRA. Root tissue density and diameter response were normalized to the control and analyzed by Dunnett's test. For an anatomy analysis, two technical replicates (another replicate was taken if the quality of the sample was low) were used to calculate the average of each biological replicate. Then, differences between treatments within each cultivar were confirmed by t-test.

Results

Impact of salinity on biomass allocation and transpiration

There was a significant reduction in the total biomass of all three cultivars under the 30 m*M* treatment ($P < 0.05$), and, for Paulsen and SO4, also under the 10 m*M* treatment ($P < 0.01$; Table 1). All three cultivars had a greater reduction in the bottom part of the roots at the 10 m*M* treatment ($P < 0.01$), while in the top part of the roots, a significant reduction was observed only under the 30 m*M* treatment ($P < 0.05$). A significant decline in stem mass was observed in Paulsen and SO4 only under the 30 m*M* treatment ($P < 0.05$) but was not observed in R-110. Leaf mass was significantly reduced in Paulsen under the 10 m*M* treatment ($P < 0.01$). However, R-110 and SO4 did not have significant changes. Consequently, root mass allocations decreased under the 30 m*M* treatment, mainly in the bottom part of the roots, with 53%, 67%, and 86% reductions in Paulsen, R-110, and SO4, respectively, compared to control. Furthermore, the top part of the root system decreased by 39%, 44%, and 49% under the 30 m*M* treatment in Paulsen, R-110, and SO4, respectively. The ratio between leaf mass and total mass increased in all three cultivars, while the stem mass ratio increased in R-110 and SO4 but did not change in Paulsen. Therefore, the ratio of root to shoot significantly decreased under the 10 m*M* treatment, compared to the control, for all three cultivars ($P < 0.05$) and under the 30 m*M* treatment only in R-110 and SO4 ($P < 0.01$; Table 1).

All three cultivars reduced their cumulative transpiration under the 30 m*M* treatment compared to control, 30% reduction for Paulsen and SO4 and 40% reduction for R-110. Paulsen and SO4 also had a significant reduction in their cumulative transpiration under the 10 m*M* treatment, while for R-110, the reduction was not significant ($P<0.05$; Fig. 1a). This pattern of reductions was similar between the 10 m*M* and 30 m*M* treatments. Paulsen and SO4 had a significant reduction, while R-110 did not. Paulsen transpired more than SO4 Under the control treatment and more than R-110 under the 30 m*M* treatment. Only R-110 increased its WUE under the 30 m*M* treatment compared to control (one-way ANOVA, $P < 0.01$; Fig. 1b). This increase in R-110's WUE is due to its relatively low reduction in shoot biomass combined with a relatively high reduction in its cumulative transpiration. SO4's WUE under the control treatment was higher than R-110's WUE and higher but not significantly than Paulsen's WUE. Under the 30 m*M* treatment, R-110's WUE increased while Paulsen's did not. Therefore, its WUE was lower than SO4's WUE.

Root system response to salinity

Root formation efficiency increased for all three cultivars, displaying a significant increase in SRA in response to salinity ($P < 0.01$). However, R-110 had a higher SRA only under the 30 m*M* treatment than the control, while

Paulsen and SO4 had a significant increase under the 10 m*M* treatment compared to the control ($P < 0.05$; Fig. 2). The root formation efficiency improved from 8.7, 7.6, and 7.6 m^2/kg, under the control treatment, to 13.8, 12, and 10.9 m^2/kg, under the 30 m*M* treatment for SO4, Paulsen, and R-110, respectively. Paulsen's and SO4's root formation efficiency also increased under the 10 m*M* treatment compared to the control, while the increase in R-110 was not significant.

Reductions of 26%, 23%, and 16% in root tissue density were observed under the 10 m*M* treatment in Paulsen, R-110, and SO4, respectively (Fig. 3). Also, under the 30 m*M* treatment, 35%, 32%, and 23% reductions were observed in Paulsen, R-110, and SO4, respectively. Furthermore, under the 10 m*M* treatment, 4%, 4%, and 16% reductions of root diameter were observed in Paulsen, R-110, and SO4, respectively, though for Paulsen and R-110, the reductions were not significant. Under the 30 m*M* treatment, 10%, 12%, and 24% reductions were observed in Paulsen, R-110, and SO4, respectively, while for R-110, it was not significant.

RLD was estimated for the entire root system and was separated based on different diameters. RLD showed that narrow roots (between 0 and 1 mm) did not substantially change in response to salinity in any of the three cultivars (Fig. 4). Paulsen also did not have significant changes between treatments in any of the root diameters (Fig. 4a), while R-110's and SO4's thick roots (between 1 and 4 mm) had significant reductions in RLD under the 30 m*M* treatment, compared to the control ($P < 0.05$; Fig. 4b, c). R-110's 1–2 mm roots' RLD significantly decreased under the 10 m*M* treatment but not between the 10 m*M* and 30 m*M* treatments. R-110's thicker roots' RLD (2–4 mm) decreased significantly only under the 30 m*M* treatment, compared to the control, while the RLD of the 3–4 mm roots did not have significant changes between treatments ($P < 0.05$; Fig. 4b). SO4's 1–2 mm roots' RLD reduced only under the 30 m*M* treatment, compared to the control, with no changes under the 10 m*M* treatment, but SO4's 2–3 mm and 3–4 mm roots' RLD was significantly reduced under the 10 m*M* and the 30 m*M* treatments, compared to the control ($P < 0.05$; Fig. 4c).

All three cultivars had changes in their root diameters, sharpening the curve in response to increased salinity levels (Fig. 5). This result indicates that thinner roots contributed more to the surface area as salinity increased. Only SO4 had significant changes between the control and salinity treatments ($P < 0.05$). SO4's D-50 and D-90 were reduced by 0.5 and 1 mm, respectively, between the control and the 30 m*M* treatment (Fig. 5c), but no significant differences were observed between the 10 m*M* and the 30 m*M* treatments. However, Paulsen and R-110 had significantly lower root diameters in the bottom part of their root system under the salinity treatments ($P < 0.05$; SI table 1), the differences in their D-50 and D-90 were not significant. The reduction of the average root diameter contributing to 50% and 90% of the cumulative surface area reached 0.14 and 0.2 mm, respectively, in Paulsen under the 30 m*M* treatment (Fig. 5a) and 0.15 and 0.37 mm in R-110 under the 30 m*M* treatment (Fig. 5b).

Root slices taken 15 mm from the root tip revealed that the stele area significantly decreased in Paulsen, but not in R-110 and SO4, under the 30 m*M* treatment, compared to the control ($P < 0.05$; Fig. 6a). The cortex area did not change (Fig. 6b), and the cortex to stele ratio significantly increased in response to salinity in Paulsen ($P < 0.05$) and R-110 ($P < 0.01$), while in SO4, the increase was close to statistically significant ($P = 0.051$; Fig. 6c). Also, the stele area, 40–50 mm from the root tip, significantly decreased in R-110 under the 30 m*M* treatment, compared to the control ($P < 0.01$; Fig. 6d). The cortex area did not change (Fig. 6e), and only R-110's cortex to stele ratio significantly increased under the 30 m*M* treatment, compared to the control ($P < 0.05$; Fig. 6f). No

significant differences were observed in root area, xylem area, and xylem density measured 15 mm and 40–50 mm from the root tip (SI fig. 1).

Cl^- and Na^+ accumulations in the roots

Cl^- accumulation in the fine roots showed a significant increase for all three cultivars as salinity increased ($P < 0.01$). Cl^- accumulation in Paulsen's, R-110's, and SO4's fine roots significantly increased in response to both salinity treatments ($P < 0.05$), but no significant differences were observed between the 10 m*M* and 30 m*M* treatments (Fig. 7a). Paulsen had 0.43, 0.55, and 0.67 mmol/g under the control, 10 m*M*, and 30 m*M* treatments, respectively. R-110 had similar results with 0.22, 0.6, and 0.68 mmol/g under the control, 10 m*M*, and 30 m*M* treatments, respectively. However, SO4 had lower levels of Cl^- than R-110 under the 10 m*M* treatment and lower than both Paulsen and R-110 under the 30 m*M* treatment, with 0.19, 0.4, and 0.49 mmol/g under the control, 10 m*M*, and 30 m*M* treatments, respectively. The fine roots' Na^+ concentration increased between the control and the 10 m*M* treatment for all three cultivars and increased between the 10 m*M* and 30 m*M* treatments in Paulsen's and SO4's roots but not in R-110's roots (Fig. 7b). SO4 had a lower root Na^+ concentration than R-110 under the 10 m*M* treatment and compared to Paulsen under the 30 m*M* treatment. All three cultivars had lower K^+/Na^+ ratios under the 10 m*M* and 30 m*M* treatments compared to control. Under the control treatment the K^+/Na^+ ratios were 2.9, 2.8 and 3.6 for Paulsen, R-110 and SO4, respectively, but with no significant differences between them (data not shown). All three cultivars had lower K^+/Na^+ ratios under the 30 m*M* treatment compared to the 10 m*M* treatment (Fig. 7c). SO4 had a higher K^+/Na^+ ratio compared to Paulsen and R-110 under the 10 m*M* treatment. Under the 30 m*M* treatment, SO4's K^+/Na^+ ratio was slightly higher but not significantly compared to Paulsen and R-110.

Discussion

SRA significantly increased in response to salinity, while root tissue density and root diameter decreased

Changes in root diameter or root tissue density can influence root formation efficiency. A reduction in the average diameter was observed in all cultivars in response to salinity (SI table 1). Similar results were shown in maize under conditions of sufficient and deficient phosphorus (Tang et al. 2019), in tomato (Lovelli et al. 2012), and in cotton (Zhang et al. 2014). However, contradictory results were also found, in which salinity caused an increase in root diameter in olive and citrus trees (Rewald et al. 2012; Tan et al. 2020).

Average root diameter is directly related to the total root surface area, mainly through the high contribution of narrow roots. While only SO4 had a significant increase in the contribution of its narrow roots to the surface area as salinity increased, Paulsen and R-110 showed the same pattern but with no significant difference between the treatments (Fig. 5). The reason for the observed alteration in response to salinity was likely due to root mortality, leading to a higher turnover rate (Snapp and Shennan 1992), with thick roots less likely to develop. R-110 and SO4 had no significant reduction in their RLD of roots thicker than 1 mm. This reduction was not linked to an increase in the total number of narrow roots (Fig. 4).

Although the increase in the narrow roots' contribution to the surface area could be a passive consequence of root death, it may be beneficial in coping with salinity. This may be the case due to the shorter lifespan of thicker roots (Hill et al. 2013), while the formation of the more efficient roots (fine roots), which have a better surface area to volume ratio, is maintained. Furthermore, as previously suggested by Neumann (1995), the reduction in root diameter, along with a reduction in root cell length and plant size, could have an adaptive benefit for increasing plants' endurance in saline conditions.

The reduction in root tissue density can aid in the metabolic investment of root formation and maintenance by reducing respiration and nutrient demands, enabling more root development for a defined metabolic cost. The observed reduction in root tissue density (Fig. 3) is, to a certain degree, associated with the increase in the cortex to stele ratio observed in Paulsen, R-110, and SO4 (Fig. 6). Under stress conditions, a large root cortical aerenchyma and a high cortex to stele ratio were found to improve plant growth (Lynch 2007; Pedersen et al. 2020; Yamauchi et al. 2019; Zhu et al. 2010). Previous experiments showed changes in the cortex to stele ratio, for example, under high salinity conditions. In two halophytes and one glycophyte species, the cortex to stele ratio increased in the halophytes while decreasing in the glycophyte (Boughalleb et al. 2009). Similarly, in citrus, in several root orders, the cortex to root ratio increased, while the stele percentage decreased in response to salinity (Rewald et al. 2012). However, tissue density was found to be related to lifespan in shoots and roots; species with low density showed a faster turnover (Ryser 1996). Consequently, reducing root tissue density may come at the cost of a higher root death rate.

Specific root length (SRL) is derived by either length or tissue density (Eissenstat 1991). In addition to length and density, SRA is also derived via root diameter, as a narrow root has more surface area per volume. More studies use SRL than SRA, yet some previous ones have shown that SRA increased in response to salinity (Hill et al. 2013; Imada et al. 2015; Shelef et al. 2010). In the current work, a significant increase in SRA was found in all cultivars. However, since there are several ways in which plants can change their strategy to cope with salinity stress, it is essential to test the mechanism. The total root length did not increase in any of the cultivars (SI fig. 2), but the average diameter and root tissue density changed. Paulsen and R-110 showed parallel patterns in which the change in tissue density was six-fold greater than the change in average diameter under 10 mM (Fig. 3). Under 30 m*M*, Paulsen showed a three-fold higher response and R-110 showed a 2.7-fold higher response in tissue density compared to the average diameter. On the other hand, SO4 showed a similar degree of change in diameter and tissue density. Thus, improvement in root formation efficiency seems to be cultivar-dependent, and its plasticity increases when salinity increases.

The importance of narrow roots to the surface area increases as salinity increases and is the result of damage to the thicker roots

Soil solution salinity is not homogeneous, and due to plants' preference for up-taking water rather than salt, salt levels can be higher near the roots (Perelman et al. 2020). Plants can react to salt heterogeneity in the soil in different ways, including enhancing root length, especially of the fine roots, which are more important for water and nutrient uptake (Cochavi et al. 2020; Rewald et al. 2011). In addition, Witzel et al. (2018) suggested that an increased number of lateral roots is related to enhanced plant performance. In the current work, none of the

cultivars had a reduction in their RLD of roots between 0 and 1 mm in diameter (Fig. 4), and no cultivar showed a significant reduction in the total RLD in the top part of the pot (SI table 1). Therefore, the lack of observed damage to the narrow roots may indicate the importance of these roots to the mechanism underlying improved salt tolerance.

A shift in biomass allocation significantly decreases the bottom part of the root system, reducing the root to shoot ratio

Salinity stress increasingly reduces root elongation as salt concentration increases (Bernstein et al., 2004). A vertical gradient of concentration usually exists in soils (Bouksila et al. 2013; Dudley and Shani 2003). Our results show that the roots in the lower part of the soil were the first to be affected by the 10 m*M* treatment in the three cultivars we tested (Table 1). These results may result from higher salinity levels at the bottom of the pot, as indicated by a higher EC in the drainage water, which was around twice the EC of the irrigation water (SI fig. 3b). Furthermore, a study done on *A. thaliana* showed that seedlings that were grown in a medium with 50 mM of NaCl significantly increased the branching of lateral roots at the top and the middle parts of the root system, while a significant reduction in root growth was observed in the bottom part (Zolla et al. 2010). Auxin is known to promote root formation in grapevines (Kracke et al. 1981), and it may also play a prominent role in specifying the location at which lateral roots form in response to salinity. Nevertheless, the physical proximity of the upper parts of the roots to the leaves may also play a role in the differences due to source-sink relationships previously shown for fruit-leaf relations (Pawar and Rana 2019).

Stems and leaves were less affected by salinity, as shown by the slight change in their dry mass, thus affecting the root to shoot ratio (Table 1). Similar reductions in root to shoot ratios in response to salinity were found in Rhodes grass under 250 mM NaCl (Céccoli et al. 2011), avocado under 15 and 25 mM (Bernstein et al. 2004), and red pepper under 100 mM (Siddikee et al. 2011). However, some previous results, in which the root system was less affected than the aboveground parts of various grapevine cultivars under 50, 100, and 250 mM NaCl, contradict these results. (Fisarakis et al. 2001; Upreti and Murti 2010). Similar results were also found in citrus under 90 mM NaCl (Rewald et al. 2012) and tomato under a range of 20 to 155 mM (Maggio et al. 2007; Munns 2002). It is possible that an increase in the root to shoot ratio reduces transpiration and, therefore, the uptake of water and salts. Or, vice versa, the reduction in the root to shoot ratio may improve the source to sink ratio for carbohydrates, in addition to keeping old leaves and stems, allowing for more storage of Na^+ and Cl^-. Our results support the latter, all three cultivars reduced root to shoot ratio in response to salinity (Table 1) alongside a reduction in cumulative transpiration (Fig. 1).

An increase in the narrow roots' contribution to the root system surface area is correlated with a higher salt exclusion capacity

As Cl^- is more present than Na^+ in grapevines irrigated with saline water (Dag et al. 2015; Fisarakis et al. 2001; Shani and Ben-Gal 2005; Walker et al. 2004), many studies have examined the capacity of different grapevine genotypes to exclude Cl^- from the leaves. It is less likely that a Cl^- excluder genotype uses its roots to store Cl^-. As

shown in Fig. 7a, SO4 had the lowest Cl^- concentration in its fine roots (28% less than Paulsen and R-110) under the 30 m*M* treatment. Another experiment supporting these findings showed that Paulsen had lower levels of Cl^- in its leaves and roots than two other non-excluder genotypes (Martin et al. 2020). Interestingly, a Cl^- excluder genotype (Ruggeri 140), compared with a Cl^- non-excluder genotype (K-51-40), had lower levels of leaf Cl^-, while in some experiments, it had higher levels of root Cl^- (Henderson et al. 2014; Walker et al. 2018), and in another, no differences were observed in the root Cl^- levels (Gong et al. 2011). Another study that examined different Cl^- excluder and non-excluder genotypes showed no difference in the entire roots Cl^- content, while the excluder genotype had lower levels in the cortical cells and slightly higher levels in the pericycle cells (Storey et al. 2003).

The differences in Cl^- exclusion capacity in grapevine cannot be entirely attributed to the transpiration stream. There were no decisive differences in cumulative transpiration between SO4 and the other cultivars (Fig. 1a). This finding supports what was previously reported, namely that Cl^- uptake occurs via ion transporters. Chlorides are transferred to the shoot mainly via cell membrane transporters, the rate-limiting factor of this process is the upload of chlorides from the root symplast to the xylem apoplast (Li et al. 2017). Similarly, a study conducted on two *Vitis* cultivars under saline conditions showed that differences in the exclusion capacity between cultivars were not due to variation in the transpiration (Abbaspour et al. 2014). In addition, another experiment, using an apoplastic tracer, showed that Cl^- entry to the roots occurs via the symplastic pathway (Gong et al. 2011). Thus, a rootstock that uploads fewer chlorides into the xylem stream is highly beneficial for growing grapevines with saline water. SO4's lower root Cl^- concentrations, combined with the above findings, support the hypothesis that grapevines' primary Cl^- exclusion mechanism operates through less symplastic uptake by the roots.

Na^+ is considered less toxic to grapevines due to their ability to accumulate it in their woody stems and roots (Munns and Tester 2008). Nevertheless, SO4 was found to exclude Na^+ from the roots better than Paulsen and R-110. Moreover, SO4's better capacity of root Na^+ exclusion did not affect its K^+ absorption. K^+ is an essential plant nutrient, it is an important cofactor in many biosynthetic processes and plays a key role in stomatal regulation (Andrés et al. 2014; Prajapati and Modi 2012), and therefore, it is essential to maintain a good K^+/Na^+ ratio. Leaf K^+/Na^+ ratio was found to be a good predictor of salinity-induced yield loss in rice (Asch et al. 2000) and tomato (Babu et al. 2012). SO4's higher K^+/Na^+ ratio under the 10 m*M* treatment may not serve as an indicator for a better salinity tolerance, but at a long range, it will delay from reaching the threshold of shoot Na^+ accumulation. SO4 is considered less tolerant to drought and salinity than Paulsen and R-110 (Corso and Bonghi 2013; Serra et al. 2014), in this experiment, SO4 showed better salt exclusion from the roots. This controversial result can be due to the combination of the excessive irrigation method we used and the shift of SO4's root system towards thinner roots. The lower hydraulic conductivity of SO4's thinner roots was less prominent than their better ion selectivity.

Conclusion

Root system formation and root efficiency are affected by increased salt levels in the soil and are genotype dependent. Roots increased their efficiency in response to salinity via a reduction in root diameter, which is a consequence of damage to the thicker roots and decreased root tissue density associated with an increase in the cortex to stele ratio. SO4 showed slightly higher root formation efficiency and lowest root salt accumulation, suggesting that fine roots have a better ion exclusion capacity and are more selective for ions than thick roots. It

is difficult to conclude whether an increase or a decrease in the root to shoot ratio can be used as a parameter for evaluating salinity tolerance characteristics in grapevine rootstocks. The ratio changes are dependent on the cultivar but also on environmental conditions, rootstock-scion combination, experimental design, and the interaction of all the above. In this work, more damage was observed in the root system in response to salinity (especially in the lower parts of the root), leading to a reduction in the root to shoot ratio.

Declarations

Funding

This research was founded by the Israeli ministry of agriculture and rural development as part of ERA-NET-5317.

Conflicts of interest/Competing interests

The authors declare no conflict of interests.

Availability of data and material

Not applicable

Code availability

Not applicable

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Yaniv Lupo, Alon Schlisser and Shuo Dong. The first draft of the manuscript was written by Alon Schlisser and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Acknowledgments

This research was part of the EnViRoS project (Opportunities for an Environmental-friendly Viticulture: optimization of water management and introduction of new Rootstock and Scion genotypes).

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Tables

Table 1 Root, stem, leaf and total plant dry mass, and root to shoot ratio. Numbers are showing mean ± standard error. Different letters represent significant differences between treatments, one-way ANOVA followed by Tukey's HSD, $P < 0.05$. ns = not significant. n = 5

Cultivar	Treatments	Bottom roots (g)	Top roots (g)	Stems (g)	Leaves (g)	Total mass (g)	Root/shoot
Paulsen	Control	29.8±3.5 a	54.1±7.7 a	54.0±4.7 a	57.2±3.5 a	195.0±14.5 a	0.75±0.05 a
	10 m*M*	14.0±2.2 b	42.0±4.0 ab	40.9±4.6 ab	54.1±2.1b	151.0±11.4 b	0.59±0.03 b
	30 m*M*	14.1±2.3 b	32.8±1.3 b	35.7±2.8 b	41.3±2.8 b	123.8±7.3 b	0.61±0.05 ab
	p-value	0.002	0.033	0.023	0.005	0.003	0.04
R-110	Control	26.7±3.8 a	56.3±7.7 a	39.6±4.5	55.8±5.8	194.2±6.6 a	0.87±0.04 a
	10 m*M*	8.0±1.2 b	42.7±5.1ab	39.9±7.2	52.3±6.9	143±18.9ab	0.57±0.05 b
	30 m*M*	8.4±1.7 b	31.4±2.5 b	38.5±3.9	48.1±1.9	126.4±8 b	0.46±0.04 b
	p-value	< 0.001	0.024	ns	ns	0.04	< 0.001
SO4	Control	26.7±3.3 a	50.9±4.5 a	57.0±4.0 a	66.5±5.2	201.1±9.8 a	0.64±0.06 a
	10 m*M*	10.9±2.4 b	41.1±3.9 a	48.9±1.8ab	62.7±4.0	163.5±8.8 b	0.46±0.03 b
	30 m*M*	3.7±1.5 b	25.7±1.8 b	37.2±4.2b	49.3±4.7	116.0±9.9 c	0.34±0.02 b
	p-value	< 0.001	0.001	0.006	ns	<0.001	< 0.001

Figures

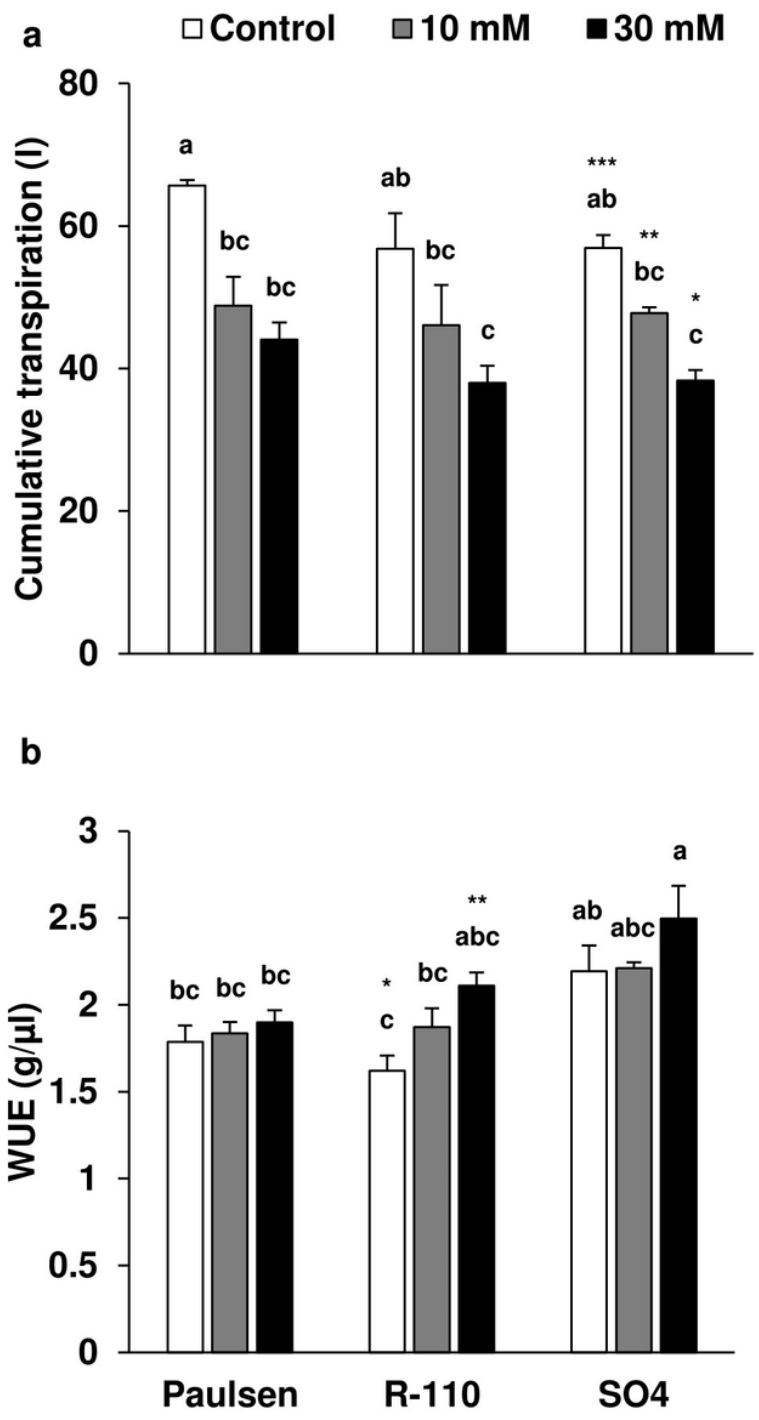

Figure 1

Cumulative transpiration and water use efficiency (WUE). (a) Cumulative transpiration. (b) WUE calculated as the total dry mass at the end of the experiment divided by the cumulative transpiration. Different letters represent significant differences between cultivars and treatments, two-way ANOVA followed by Tukey's HSD, $P < 0.01$. Asterisks represent significant differences between treatments within each cultivar, one-way ANOVA followed by Tukey's HSD test, $P < 0.01$. Bars represent standard error. n = 3–5

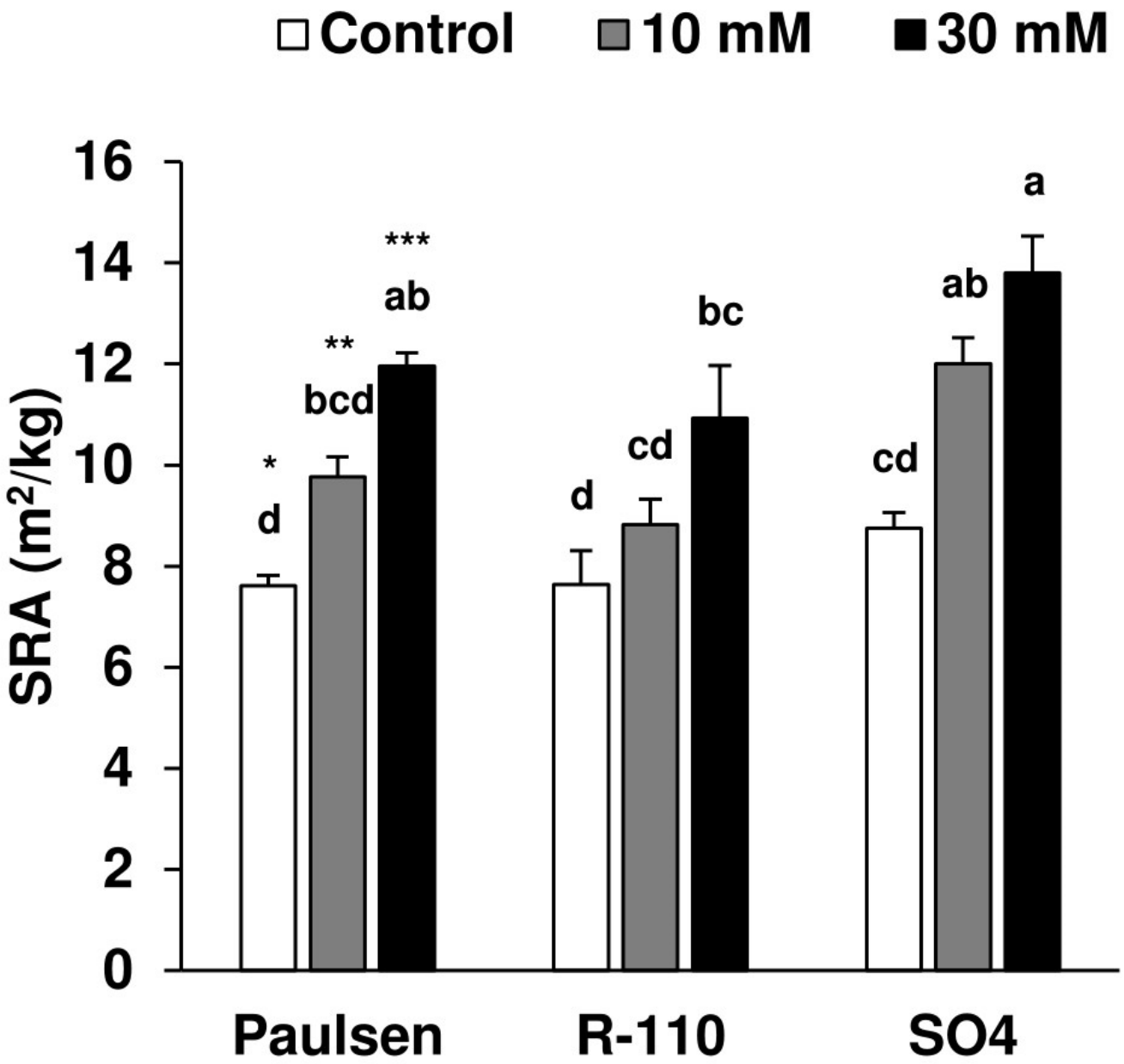

Figure 2

Specific root area (SRA) of the whole root system. Different letters represent significant differences between cultivars and treatments, two-way ANOVA followed by Tukey's HSD, $P < 0.01$. Asterisks represent significant differences between treatments within each cultivar, one-way ANOVA followed by Tukey's HSD test, $P < 0.05$. Bars represent standard error. n = 5

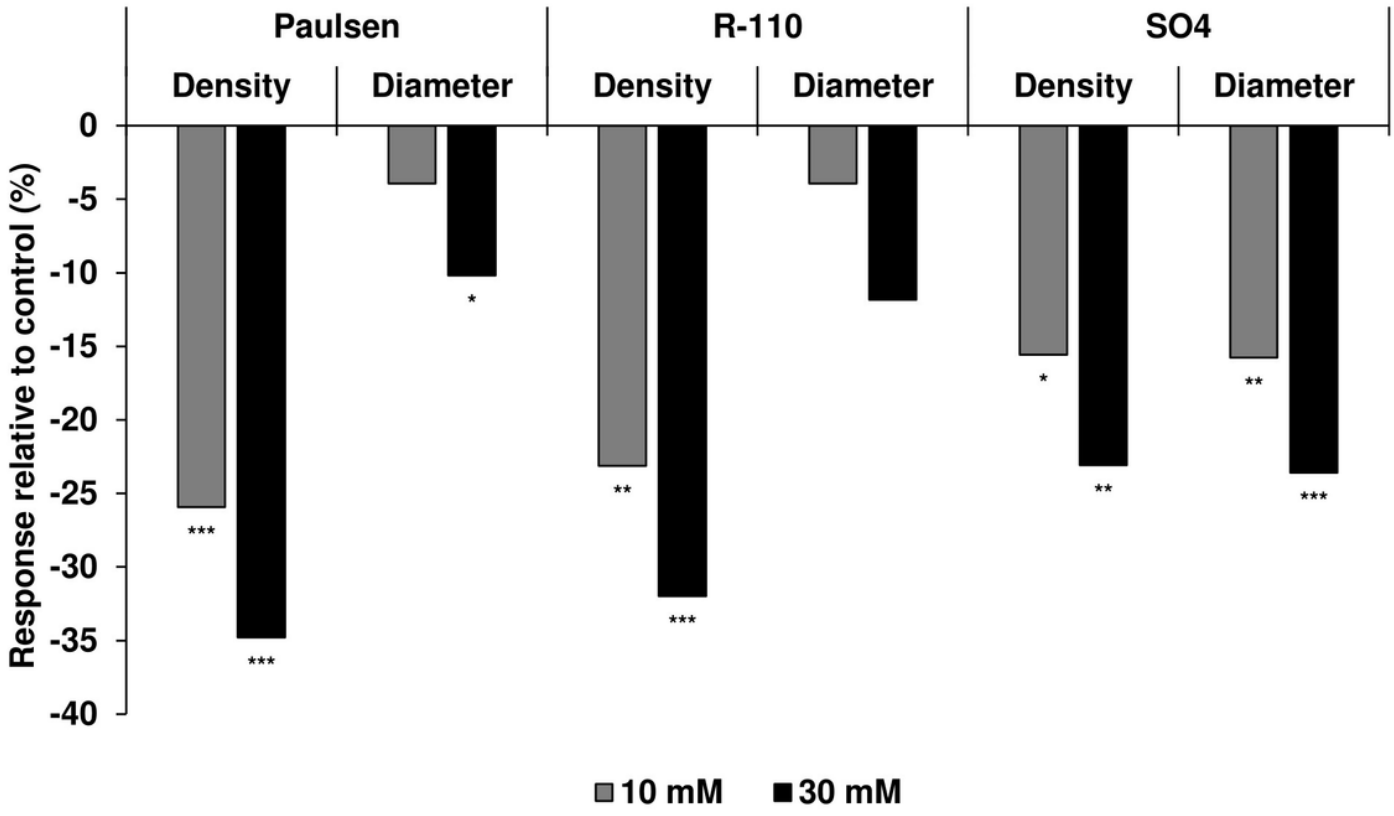

Figure 3

Root density and diameter response relative to the control treatment. Asterisks represent significant differences between the salt treatments and the control treatment. *P < 0.05; **P < 0.01; ***P < 0.001, Dunnett's test. n = 5

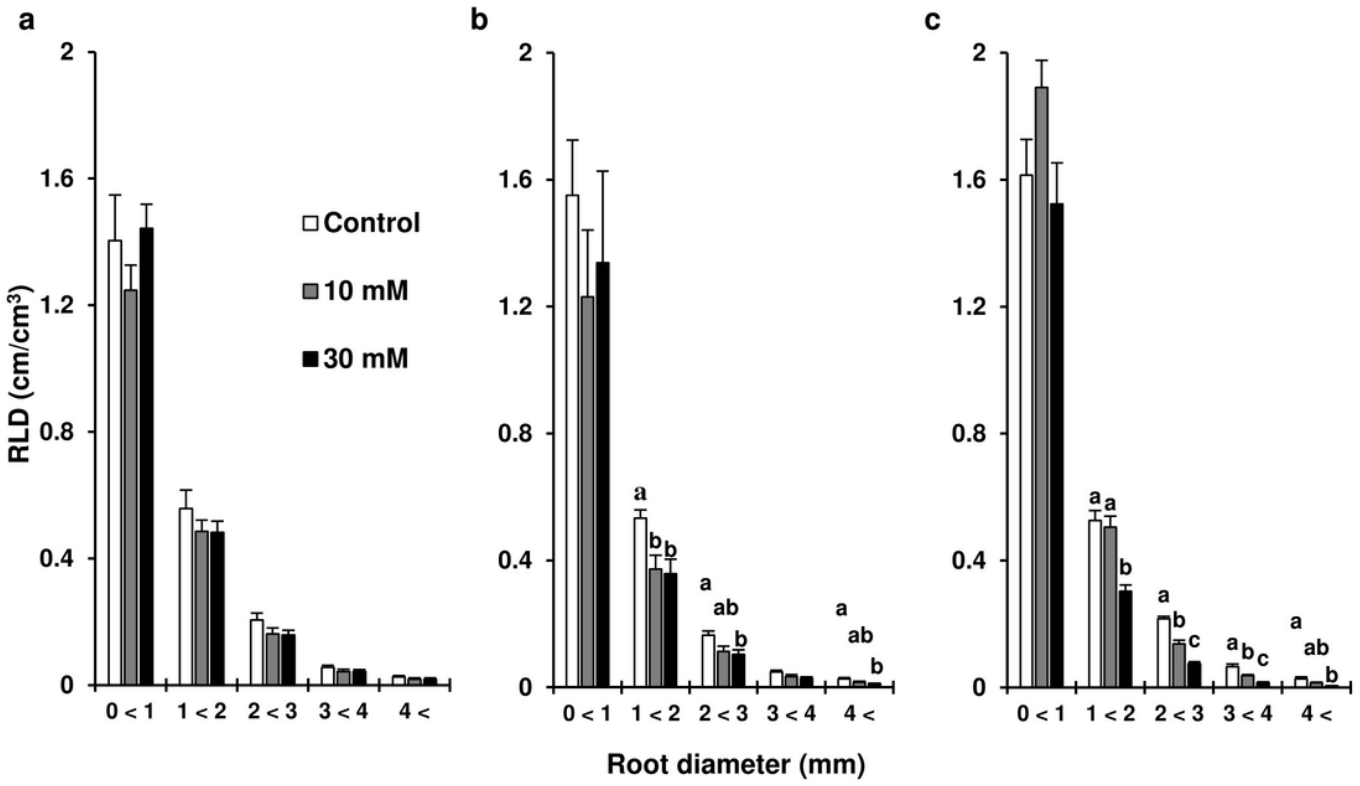

Figure 4

Root length density (RLD) of (a) Paulsen, (b) R-110 and (c) SO4, calculated by the ratio of the root length (cm) per volume of soil (cm3) for different root diameters. Different letters represent significant differences between treatments, one-way ANOVA followed by Tukey's HSD test, $P < 0.05$. Bars represent standard error. n = 5

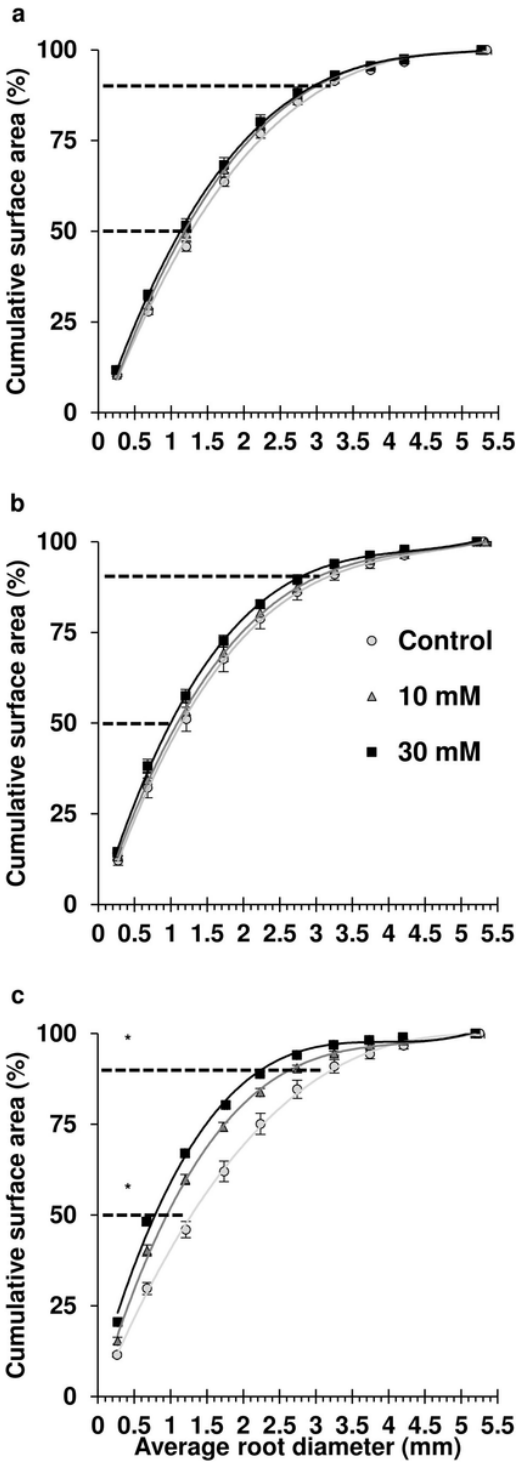

Figure 5

Root system's cumulative surface area (as a percent of total) contributed by different root diameters of (a) Paulsen, (b) R-110, and (c) SO4. Lower and upper dotted black lines represent D-50 and D-90, respectively. Asterisks represent significant differences in D-50 or D-90 between treatments, one-way ANOVA followed by Tukey's HSD test, $P < 0.05$. Bars represent standard error. n = 5

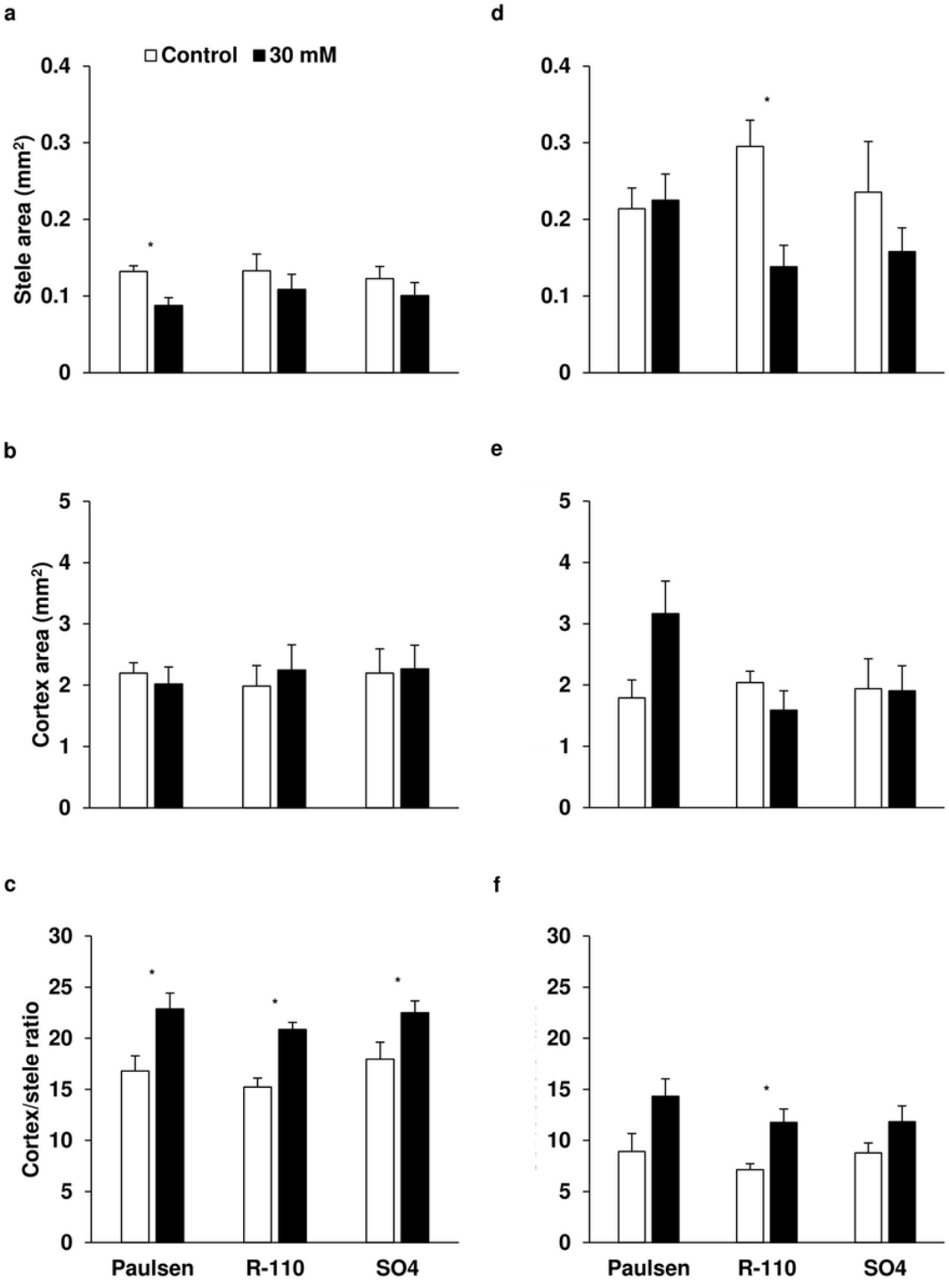

Figure 6

Stele area, cortex area, and cortex to stele ratio measured 15 mm from the root tip (a, b, and c) and 40–50 mm from the root tip (d, e and f). Asterisks represent significant differences between treatments within each cultivar, Student's t-test, $P < 0.05$. Bars represent standard error. n = 6

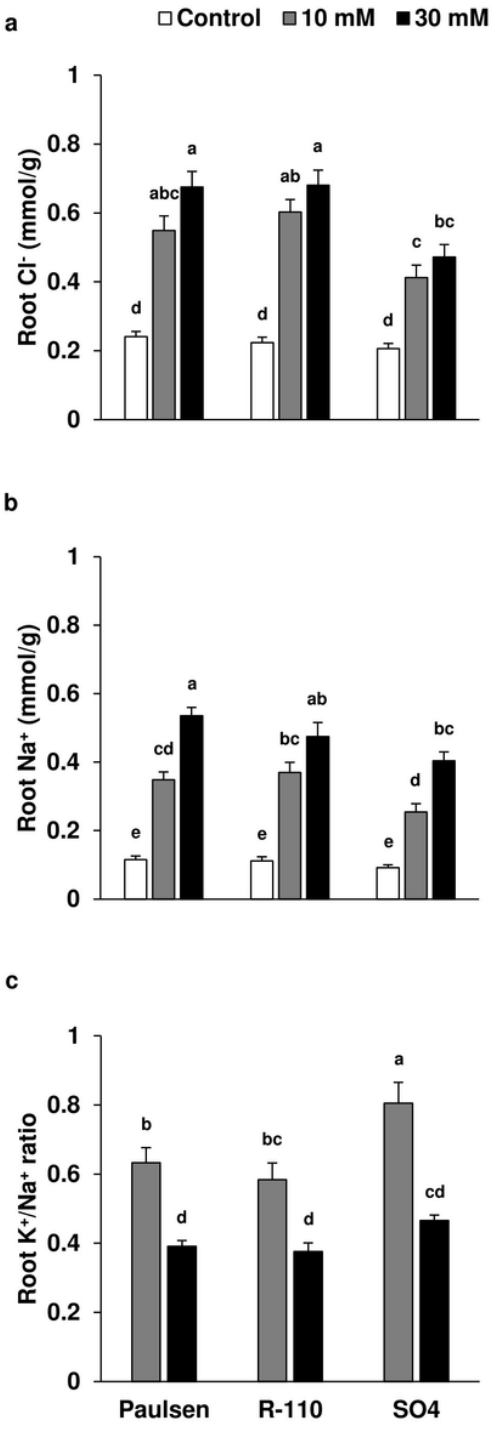

Figure 7

Roots’ chloride (a) and sodium (b) concentrations and potassium to sodium ratio (c). Different letters represent significant differences between cultivars and treatments, two-way ANOVA followed by Tukey's HSD test, $P < 0.05$. Bars represent standard error. n = 6

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementary.pdf