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ABIOTIC STRESS RESISTANCE OF SEVERAL TOMATO LANDRACES FROM SALINE ZONES OF BIHOR COUNTY

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Abstract

A major constraint to global food production is the selection of crops that are better adapted to resource limited environments and soil conditions. In the past decades, the research in this field has focused on the study of the effect of abiotic stress on plants and elucidating signalling pathways that govern the appropriate and coordinated response to abiotic stress. This study identified some tolerant tomato landraces to salt and drought stress from saline zones of Bihor County, in North-Western Romania. Phenotypic characterization of collected landraces provided information regarding drought and salt stress resistance compared with the 'Marmande' cultivar. Root density, leaf area and the reaction under different concentrations of salt and different water content were compared to conclude which landraces are more suitable for future genotypic studies in order to use them in breeding programs. The aim is to obtain genotypes with increased drought and salt stress resistance to improve crop yield and quality in saline environments. This study highlights the potential of local tomato landraces from Bihor County as valuable genetic resources for developing resilient varieties that can thrive in challenging environmental conditions.

Key words: *Solanum lycopersicum L., Marmande cultivar, drought stress, salt stress, leaf area, root density.*

INTRODUCTION

According to recent estimates by the United Nations Food and Agriculture Organization, cereal production must double before 2050 to meet the world's expanding population's demand for food and the growing competition for crops, considering their use as sources of fibre, bioenergy, and other industrial uses (www.fao.org).

A majority of the Earth's arable land is affected by salinity, due to the expansion of drought and excessive use of agricultural land. All these are due, mostly, to the increase in global temperature and climate variability (Dolferus et al., 2014). The development of new crop plant varieties with enhanced resistance to abiotic stresses (i.e., heat, drought, salinity, and UVB rays) is a major concern on a global scale (Nowicka et al., 2018; Martinez et al., 2018; Machado R.M.A. et al, 2017, Ors et al, 2017)). These varieties or local landraces make it possible to grow them on soils already affected by increased salinity or drought (Broccanello et al., 2023), ultimately increasing food security and sustainability in regions facing these challenges. By incorporating traits that improve resilience to these stressors, farmers or breeders can continue to develop crops in areas that were previously unsuitable for cultivation.

It is believed that phenotypic plasticity rather than genetic diversity may prove to be crucial for plants to thrive in specific surroundings amid swift changes in the environment (Vitasse et al., 2010; Gratani, 2014). Under harsh circumstances like cold, drought, and salt, the genotypes cultivated under different environmental conditions may demonstrate phenotypic plasticity (Tester and Langridge, 2010). Therefore, there is a need for quantitative analyses of plant traits to accelerate the selection of crops that are highly adapted to resource-limited environments and soil conditions, which is also a significant barrier to the production of food worldwide. (Fiorani and Schurr, 2013).

Bihor County, located in the north-west of Romania, has an important area of soil affected by salinization. From the total of polluted soils of the county, charted during the period 2013-2017, 9,11% of the surface is affected by different degrees of salinity: weak (38122 ha), moderate (900 ha), strong (400 ha) (http://anpmbh.ro). Tomatoes (*Solanum lycopersicum* L.) are one of the most valuable and economically significant vegetables grown worldwide for their fruits, and their productivity is steadily declining due to their vulnerability to abiotic stresses (Raja et al., 2020). Developing strategies to mitigate the impact of drought and salt stress on tomato plants is crucial for maintaining high yields and ensuring food security. Implementing innovative agricultural practices, such as precision irrigation and genetic engineering, can help improve tomato resilience to these challenging environmental conditions (Polenta et al, 2020). The purpose of this study is to identify some tolerant tomato landraces to salt and drought stress, analyse the synergistic effect of salinity and drought stress on plant growth and development and their use for initiation of a breeding program in order to obtain resistant hybrids, with tolerance to salinity and stable production (Sahin et al., 2018), Cui et al. (2020), Arshad et al. (2023).

Phenotyping platforms using non-invasive technologies have achieved strong development in the last decades Pieruschka et al. (2019). For simulation and measuring the effect of environmental conditions on tomato plants in a greenhouse a high throughput installation, Phenotyping Platform HAS-R(S) SDS was used.

In the present study, early plant growth was subjected to several forms of abiotic stress, including drought stress, salt stress, and a combination of the two (Ors et al., 2017; Ors et al., 2021). In terms of comparison, a conventional cultivar was subjected to the same treatment. Under two salt concentrations (0.2%, and 0.3%) and soil water content (60%, 20%), leaf area and water consumption were studied.

MATERIALS AND METHODS

Experimental design and stress treatments

For the experiment 4 different tomato varieties were used, collected from local farmers in Bihor County. The landraces were designated after the location: 'Ateaș 136', 'Ateaș 37', and 'Cefa 7'. 'Marmande' is the cultivar used for control. The seeds were sown in pots containing a mixture of 50% sandy (Maros) soil and 50% Terra peat soil. Fertiliser was added to each pot in equal quantity (SUBSTRLAL®, Osmocote Plus®, Wals-Siezenheim, Austria). After sprouting, the plants were grown in a 16/8 hour light/dark programme until they reached the first layer of true leaves when the water capacity of the soil was assessed. Within the group of plants for drought stress experiment, water was limited to 20% of the total soil water capacity and the control group of plants was normally watered to 60% of the total soil water capacity. All the pots of the experiment were watered automatically by a phenotyping platform moving system that had a balance connected to a computer-controlled peristaltic pump. A radio-frequency identifier was added to each pot. The plants were grown in 8 replicates and the plantlets were exposed to 4 different experimental conditions.

The plants were watered according to the preset watering protocols, and the growth of their above ground, green leaf and shoot area was monitored by digital photography. The measurements were made at an interval of seven days.

Plant Phenotyping

Leaf area, water consumption and root density measurements were performed on the Phenotyping Platform HAS-R(S)SDS (Shoots/Roots Stress Diagnostic System) at the Biological Research Centre of Hungarian Academy of Science, Szeged, Hungary, as described by Cseri et al. (2013), Kondić-Špika et al. (2022) and Dénes et al. (2023). The platform monitored the plant growth by digital photography using an Olympus C-7070WZ (Olympus Ltd., Southend-on Sea, UK) digital camera. The photographs of the plants were made from different sideways positions due to multistep rotations of the plant pots and the semi-automated system provided data for each individual plant in terms of leaf area and water consumption. The leaf area was automatically determined using the images from where the background was subtracted and the remaining green pixels were counted. The water consumption parameter was calculated from the weight measurement of the pots before watering. The data regarding the development of the roots was obtained from minirhizotrons by photographing the transparent pots (cylinders) from different side views and bottom. The growth of the plants was monitored for 3 weeks. The Matlab software tools with the Image Processing Toolbox Ver. r2013b (The MathWorks Inc., Natick, MA, USA) was used to analyse the data from the phenotyping platform (Figure 1).

Figure 1. Tomato plants with the first layer of true leaves. Day 1 of the experiment

Statistical analysis

The measurements were done on 8 plantlets from each landrace and standard cultivar. The parameters from the performed treatments were assessed using a one-way ANOVA. For data analysis, the OriginPro 8 data processing program was used.

RESULTS AND DISCUSSIONS

The studied tomato landraces were treated with 0.2% and 0.3% salt for 21 days and the leaf area, the water consumption and the root density were measured by the phenotyping platform.

In the experiment with 0.2% salt treatment the leaf area measurements for 21 days (Figure 2) under normal watering (a) and under water limitation (b) were plotted. In the normal watering conditions (panel a) there is a minor difference between control plants (with no salt treatment, solid symbol and line) and 0.2 % salt watered plants (open symbol, dotted line). It can be observed that 'Cefa7' landrace (solid and open orange diamond) and commercial cultivar 'Marmande' (solid and open pink down triangle) reacted to the salt treatment evidenced by the reduced leaf area (open orange diamond and open pink down triangle, respectively), whereas 'Ateas136' (open up blue triangle) and 'Ateas37' (open green circle) presented increased leaf area as compared to the control plants that were not subjected to salt stress (solid blue triangle and solid green circle, respectively) by day 14. In the case of 'Ateaș 37' (green circle) on day 7 of the treatment the treated plants (empty green circle) presented the same leaf area as the control plants (solid green circle), whereas starting from day 14, the leaf area of the treated plants increased. In the case of 'Ateaș 136' (blue up triangle), on day 7 and day 14, the leaf area of the treated plants (blue open up triangle) slightly decrease; however, from day 14 onward, the measured leaf area of the treated plants started to increase as compared to plants that were not subjected to salt stress (blue solid up triangle); in the day 21 the leaf area of treated plants is bigger than control. Previous studies have also reported that plants subjected to multiple abiotic stresses adversely affect their development, including leaf area (Ors & Suarez, 2017). Physiological responses of cabbage to different levels of salinity and drought proved to have a negative impact on the development of tomato seedlings. When applied independently, drought and salt stress have had a detrimental impact on plant growth, including fresh weights of the roots, shoots, and roots. However, the negative effects of each stressor were amplified when drought and salinity were combined (Ors et al., 2021). This outcome has also been observed in multiple crops, including barley, onion (Hanci & Cebebci, 2015), tomato (Al-Omran, A. M., Al-Ghobari, H., & Alazba, A., 2004) and squash (Abd El-Mageed, T. A., & Semida, W. M., 2015).

Regarding the water restricted conditions (panel b) it can be observed that there is a slight difference between the control plants (solid symbol) as compared to the salt treated plants (open symbol). The plants subjected to salt stress presented reduced leaf area by up to two-fold between days 7 and 14 as compared to the control plants that are grouped together (i.e., in the upper part of the figure, panel b, Figure 2).

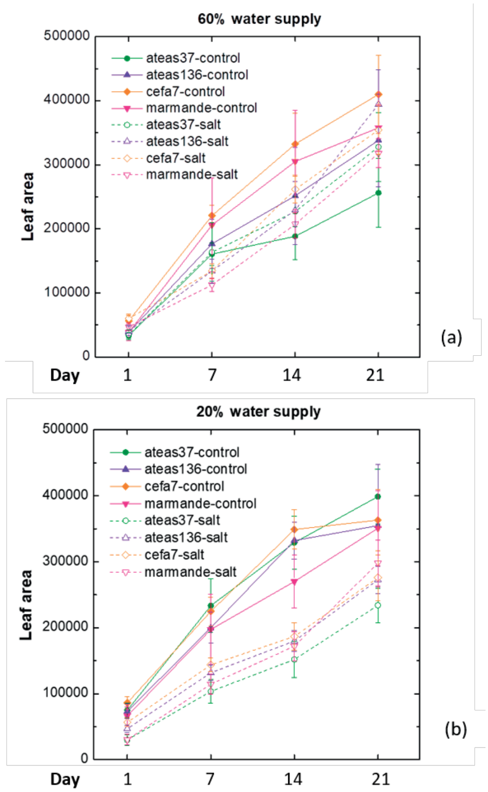

Figure 2. Measurements of leaf area under control and 0,2% saline stress in normal watered (panel a) and drought stress (panel b); for each landrace 'Ateaş37' (circle), 'Ateaş136' (up triangle), 'Cefa7' (diamond) and (solid line and symbol) and salt added substrate standard cultivar Marmande (down triangle), leaf area measurements of plants grown in normal (dotted line, open symbol)

In Figure 3 the leaf area measurements at the last day of treatment are presented and each salt-stressed landrace compared with its control can be visualised. It can be observed that under water restricted conditions 'Ateaş 37' presented significantly reduced leaf area compared with the other landraces and the control cultivar 'Marmande'. Conversely, under normal watered conditions, the most elevated leaf area has been observed in 'Ateaş 136'.

In the tomato experiment, we highlighted that the presence of 0.2% salt in the soil did not induce significant differences in the growth of plants under well watered conditions (60% soil water content). On the other hand, the presence of salt induced a 20-40% decrease in green shoot/leaf area (Figure 2b) under water limited conditions (20% soil water content). Assimakopoulou et al. (2015) evaluated the responses of several cultivars and hybrids of cherry tomatoes to different salt concentrations and revealed that the growth inhibition proved to be due to the toxicity of Na^+ and Cl^- ions and imbalances at nutritional levels impacted by salt stress. In a different study, at a degree of salinity of 5.5 dS m^{-1}, the cultivar Raf's leaf area was recorded to be around 2700 cm^2, however at 11 dS m^{-1}, the foliage was reduced in dimensions and their area drastically dropped to 1800 cm^2 (Sánchez et al., 2010). Salinity-induced morphological alterations, including plant height, dry matter (%), leaf area, and fruit count/plant in tomato cultivar PKM 1 were evaluated. Tomato plants suffered adverse effects from treatment with water containing NaCl at multiple concentrations of 25, 50, 100, 150, and 200 mM for 90 days after seeding. For instance, it was discovered that the 200 mM NaCl treatment decreased the number of fruits per plant to 4 from 15 in the control and the plant leaf area by 43.91%. Furthermore, the plants were 76.17 cm smaller than the control at this dosage (Babu et al., 2012).

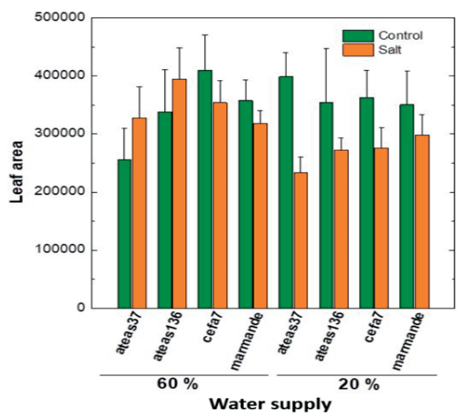

Figure 3. Leaf area measurements of control and 0.2% salt stress samples on the last day of treatment under different watering regimes. Data shown are expressed as means of 8 independent measurements ± SD

Numerous other researchers have demonstrated that morphological alterations were all affected either negatively or favourably by the salt variation in the growth medium. The findings of certain studies that evaluated the morphological alterations in tomato cultivars under salt stress have been reported in this regard (Maeda et al., 2020; Tanveer et al., 2020; Parvin et al., 2016). One adaptive morphological method to prevent water loss through transpiration may be to reduce plant height, leaf area, number, and length when exposed to salt stress. The toxicity of Na^+ and Cl^-ions that build up in cells, which inhibit the development of new leaves, could possibly constitute the root of the problem.

The water consumption measurement (Figure 4) in the case of normal watering (panel a) shows an evolution for 'Marmande' (solid and open pink down triangle) and 'Cefa7' (solid and open orange diamond), with bigger water consumption for the salt treated plants (open pink down triangle and open orange diamond) compared with control plants (solid pink down triangle and solid orange diamond) for the first 14 days and in day 21 the water consumption equalises for both salt treated and control plants.

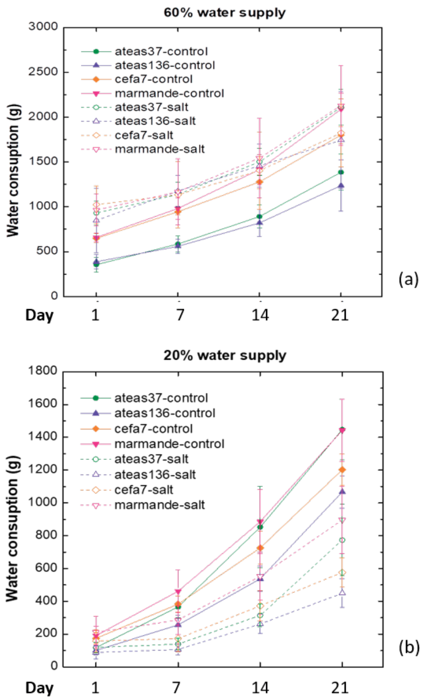

Figure 4. Measurements of water consumption under control and 0,2% salt stress in normal watered (a) and drought stress (b); for each landrace plants grown in normal (solid line and symbol) and salt added substrate (dotted line, open symbol)

For 'Ateaş37' (solid and open green circle) and 'Ateaş136' (solid and open blue up triangle) the salt treated plants (open green circle and open blue up triangle, respectively) show a bigger water consumption than control plants (solid green circle and solid blue up triangle, respectively). When water limitation is imposed (Figure 4, panel b), it is observed a lower water consumption for salt treated plants (open symbols) than the untreated control plants (solid symbols) for all the tomato landraces studied. This difference between control (solid lines) and treated plants (dotted lines) evolves proportionally with time.

End point water consumption measurements on the last day of salt treatment (Figure 5) under normal water showed little to no difference between 'Marmande' and 'Cefa7' control (green columns) and treated plants (orange columns) and a significant level of water consumption in treated plants particularly for 'Ateas37' and 'Ateaş136' under the same regime. When water consumption was measured under drought stress, all the studied landraces showed an elevated consumption for control plants (green columns), almost 2 times bigger as compared to the 0.2% salt treated plants (orange columns); however, lower water consumption was noticed for control drought stress plants as compared to normal water regime control plants.

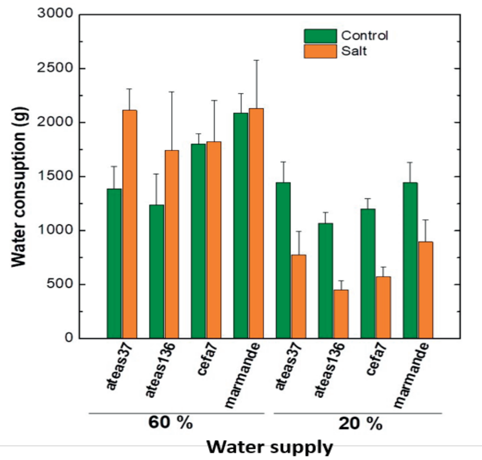

Figure 5. Comparison of leaf area measurements for control and 0,2% salt stress samples on the last day of treatment under different watering regimes. Data shown are expressed as means of 8 independent measurements ± SD

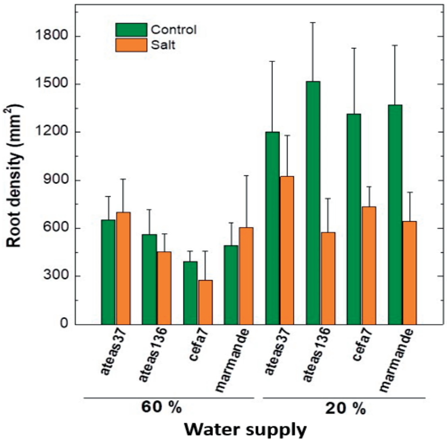

Figure 6. Comparison of root density measurements for control and 0,2% salt stress samples at the last day of treatment under different watering regimes. Data shown are expressed as means of 8 independent measurements ± SD

Root density measurements under 0,2% salt treatment show a decrease compared with the non-treated plants. Under salt and drought stress combined, the root density of treated plants is decreased compared with no salt treated plants, but there is an increase in root density compared with normally watered plants (control and salt treated). 'Ateaș37', under a normal watering regime and with 0.2% salt treatment, was the only landrace that presented an increase in root density (Figure 6). It was demonstrated for certain crops (rice, tomato) that under mild salinity the plants tend to modify their toot architecture system developing deeper roots with fewer lateral roots in this way maximising the opportunity to access deeper soil with more water and less salinity (Ijaz et al., 2019; Gandullo et al., 2021; Shelden and Munns, 2023)

In the 0.3% salt experiment, the leaf area (Figures 7 and 8) and the water consumption (Figures 9 and 10) were measured under normal watering (panel a Figures 7 and 9) and drought stress (panel b, Figures 7 and 9). The leaf area measurements under normal water supply (Figure 7 panel a) showed a greater value with regard to the control plants (solid symbol) as compared to the salt treated plants (open symbol), with the smallest variation during the 12 days measurements for 'Marmande' (pink down triangle). Thus, the 'Marmande' cultivar in day 21 presented leaf area measurements approximately equivalent to those identified in the control plants, with the most significant variation in 'Cefa7' (orange diamond).

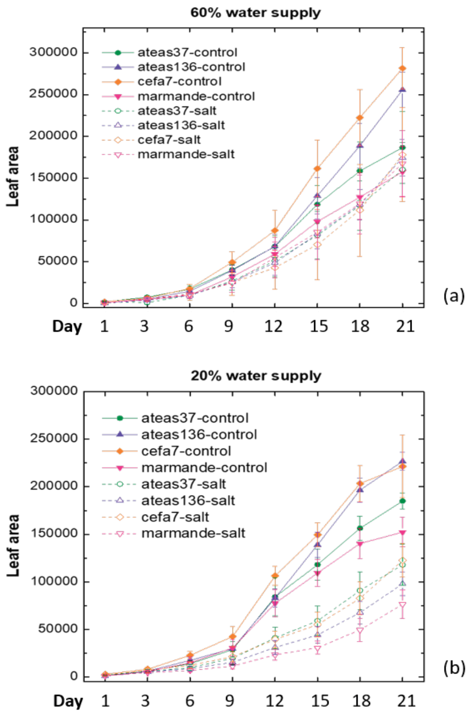

Figure 7. Leaf area measurements of control and 0,3% salt stress in normal watered (a) and drought stress (b); for each landrace plants grown in normal (solid line and symbol) and salt added substrate (dotted line, open symbol) are shown

When drought stress is applied jointly with salt stress (Figure 7, panel b), the leaf area slightly decreased till day 9 and continuing its downward till day 18, when the difference between the control (solid line and symbol) and treated plants (dotted line and open symbol) is revealed to be three times greater.

At the end of the experiment, leaf area measurements (Figure 8) under salt stress only, the leaf area of 'Marmande' proved to be similar in both control (green columns) and stressed plants (orange columns), whereas under both stresses (i.e., salt and drought), the leaf area of the treated plants (orange column) significantly decreased approximately at half the value.

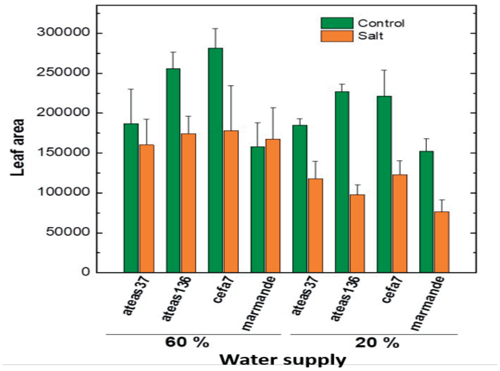

Figure 8. Leaf area comparison of control and 0.3% salt stress samples at the end of the experiment under different watering regimes. Data shown are expressed as means of 8 independent measurements ± SD

In the case of 'Ateaș37', under salt stress only, the leaf area values of treated plants were 15% smaller compared to the control plants. When both stresses were applied, salt and hydric, the values of stressed plants decreased up to 40%. For 'Ateaș136' and 'Cefa7' the salt stressed plants and the salt and drought stressed plants presented lower values for stressed plants, whereas the values in the combined stressed plants were significantly lower as compared to both the controls and their counterparts with only salt stress.

Regarding the water consumption (Figure 9), when 0.3% salt stress was applied, the 'Marmande' commercial cultivar showed a major difference between control (solid pink down triangle, solid pink line) and salt treated plants (open down triangle, dotted pink line), whereas the other studied landraces evolved together with minimum differences between control (solid symbol and solid line) and treated plants (open symbol and dotted line).

When the water consumption is compared at the end of the experiment (Figure 10), the control plants utilise water better than salt stressed plants and, in the case of double stressed plants, (salt and drought together) the water intake of control plants is considerably lower than control salt stressed only plants whereas the measurements for double stressed plants are extremely low.

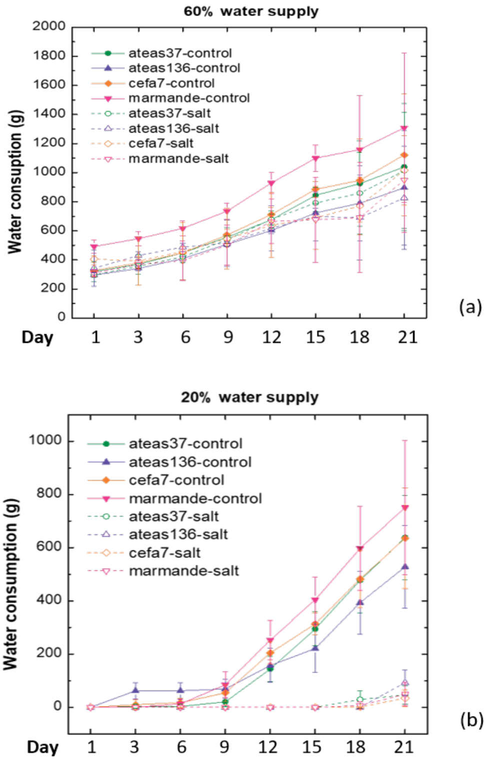

Figure 9. Influence of water supply on water consumption under control and 0,3% salt stress in normal watered (a) and drought stress (b); for each tomato landrace

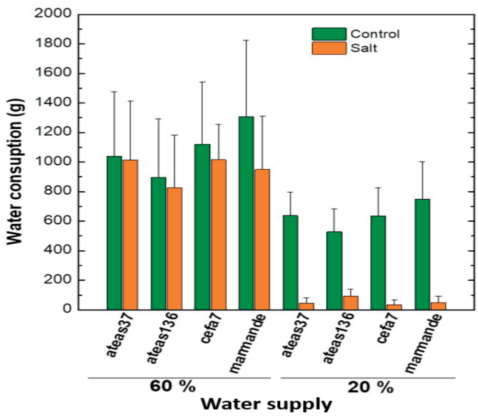

Figure 10. Comparison of control and 0,3% salt stress samples at the end of the experiment under different watering regimes. Data shown are expressed as means of 8 independent measurements ± SD

In the study conducted by Reina-Sánchez et al., 2005, tomato plants cultivated in particularly salinized environments used 40% less water than control plants. The salinity of the nutrient solution accounted for nearly all of the variances in plant water absorption, and the connection among salinity and water uptake was linear. This indicates that plants can modify their water uptake to saline conditions, which could potentially conserve water resources in arid situations (Wan et al., 2007). In order to maximise water, use in agriculture, more studies may also examine how various plant species react to differing salt levels, as correlations between water uptakes can also be cultivar-specific.

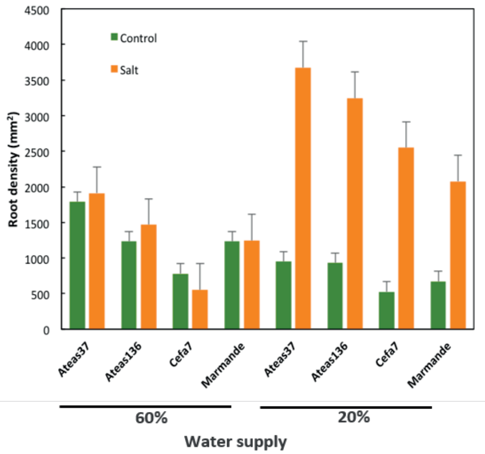

Figure 11. Comparison of root density measurements for control and 0,3% salt stress samples at the last day of treatment under different watering regimes. Data shown are expressed as means of 8 independent measurements ± SD

Root density measurement under a normal watering regime showed a slight increase for 'Ateaş37' and 'Ateaş136' compared with no salt treated controls and a small decrease for 'Cefa7'. Control cultivar 'Marmande' had the same root density in control plants and 0,3% salt treated plants. When both stresses (salt and drought) were applied, the root density of the treated plants exhibited a 4-5 fold increase as compared to their controls that presented smaller root density than the normal watered control plants. This extended root system of the tomato plants grown in saline soil is in line with the research of Anqi et al. (2020) that showed a broad root system contributes to decrease in soil salinity and crops with bigger root systems coop better with increased soil salinity.

CONCLUSIONS

It was noteworthy to observe that the presence of 0,3% salt in the soil seriously limited the water uptake. On day 9, the leaf area measurements of the 'Marmande' cultivar were roughly similar as those observed in the untreated plants, with 'Cefa7' showing the greatest variation. When salt stress and drought stress were jointly applied, the leaf area gradually diminished until day six and continued to decline until day eighteen, when the difference between the treated plants (dotted line and open symbol) and the control (solid line and symbol) turned out to be three times larger. Therefore, preeminent tolerance against the effect of salt was observed in the case of 'Cefa7' landrace and 'Marmande' cultivar. The findings suggest that these landraces have the ability to adapt and thrive under combined drought and salt stress, making them promising candidates for breeding programs aimed to improve crop resilience in harsh environments. Ultimately, utilising these local tomato landraces could contribute to sustainable agriculture practices and food security in regions prone to environmental stressors.

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