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Research Article

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The Impact of Indigenous and Selected Mycorrhizal Species on Citrus Rootstock Salinity Stress Resistance

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ABSTRACT
Plants encounter numerous abiotic and biotic stresses throughout their lives, such as drought, low temperature, disease, and salt stress. Of these stresses, salinity is one of the most common abiotic stresses that pose a threat to agricultural production worldwide (Ortas *et al.*, 2021). Salt stress poses a major problem for plant production on the ground surface. Salinity causes physiological and biochemical imbalances that affect plants' growth and productivity. Citrus plants are very sensitive to salinity. After summarizing the current knowledge on salt effects on plant growth and the role of AMF in increasing salt tolerance of citrus rootstocks, the main objective of the research was to determine the effect of natural and selected mycorrhizal inoculation on growth and nutrient uptake of citrus rootstocks. This experiment was carried out in the greenhouse of the Faculty of Agriculture, Soil Science and Plant Nutrition Department at Çukurova University. This experiment consisted of two types of citrus rootstocks (Citrus reshni/Cleopatra species and C35 species), three types of mycorrhizal treatments (control, natural/Indigenous and *Rhizophagus clarus)* and half of the soil was subjected to sterilization. Four parameters SPAD, Photosystem II (PS II), length and diameter were studied. JMP computer program was used to analyze the data for which Tukey's test ($p<0.05$) and Student t test were employed to compare means. The findings showed that C35 species were more resistant to salt stress than Cleopatra species. Plants grown in non-sterile soil perform better than the plants grown in sterile soil.
Keywords: Salinity, citrus, Mycorrhiza, climate change

INTRODUCTION

Plants face numerous abiotic and biotic stresses throughout their life, such as drought, low temperature, disease and salt stress. Out of these stresses, salinity is one of the most common abiotic stresses that pose a threat to agriculture worldwide (Ortas et al., 2021). Salt stress is a major problem for agriculture because it reduces the amount of water and essential elements available to plants causing physiological and biochemical imbalances that affect their growth and productivity. The main cause of salinity is natural processes or man-made developments. As a result of these processes, dissolved salts accumulate in the soil water, affecting plant growth (Parihar and Bora, 2019). Since salinity and salt stress have a great negative impact on crops, it is becoming one of the most important abiotic stresses impacting the productivity and growth of citrus plants, which are widely grown in tropical and subtropical regions. Citrus plants are very sensitive to salinity and their adaptation to saline environments needs to be improved to increase their productivity and quality. Since citrus plants are sensitive to salt stress, they show reduced water and nutrient uptake, photosynthesis and biomass accumulation under saline conditions. (Oppenheimer, 1937) was one of the first research to study the effect of salt water on citrus rootstocks. Like many woody fruit plants, citrus can accumulate toxic levels of Cl-, Na+ or both in plant tissues (Levy and Boman, 1990). Citrus rootstocks show symptoms such as leaf chlorosis, necrosis, defoliation, reduced photosynthesis and reduced biomass when exposed to salt stress. There is a wide genetic diversity in citrus that provides a variety of materials that can serve as abiotic stress-resistant rootstocks (Cimen and Yeşiloglu, 2016). When rootstocks are used in fruit production, they exhibit greater tolerance to abiotic stressors such as salinity, heavy metals, nutritional stress, water stress, and alkalinity in addition to a stronger resistance to infections. The reduction in photosynthesis due to salt stress in citrus can inhibit growth and lead to fall and winter defoliation. The cumulative detrimental effect of salinity can cause Cl- levels above 20 g kg-1 leaf dry weight and significantly reduce yields after two to three years, particularly in species with strong yields like grapefruit (Levy and Shalhevet, 1990). Salt stress is a particular problem for citrus rootstocks which are the lower part of grafted citrus plants, providing support and anchorage to the scion (the upper part that bears the fruit). Cl-, not Na+, is responsible for citrus rootstocks' sensitivity to sodium chloride in terms of growth decrease and leaf burn symptoms. (Zekri and Parsons, 1992). Salt stress also impacts the quantity and quality of fruit produced by the scion. Therefore, it is really of great importance to select salt-tolerant rootstocks that can cope with saline conditions and protect the scion from salt damage. Salt stress has also been reported to reduce total plant biomass by 27-38% (Iglesias *et al.*, 2004). Some studies have proved that citrus plants are susceptible to various diseases and pests when they are exposed to salt stress. (Nayem *et al.*, 2020) research conducted by the University of Turkey revealed that the development of citrus scab weevil disease is accelerated by salt stress. Considering all this information, it is necessary to develop several soil plant mechanisms for healthy citrus cultivation against salt stress. In- order -to develop these mechanisms, utilizing nature and the ecosystem is extremely important for a sustainable ecosystem and agriculture. One of the strategies to increase the salt tolerance of citrus plants is to use arbuscular mycorrhizal fungi (AMF), the largest symbiotic microorganisms that colonize the roots of most terrestrial plants (Ortas, 2012). Mycorrhiza, by definition, requires a symbiotic relationship between plant roots and fungi which can improve plant nutrient and water uptake, osmotic adjustment, antioxidant defense and hormonal balance under salt stress. Arbuscular mycorrhizal (AM) fungi are crucial mutualistic symbionts of the majority of plant species, with essential roles in plant nutrient uptake and stress mitigation (Větrovský *et al.*, 2023). Mycorrhizae have attracted interest as one of the microorganisms that increase a crop's salt stress tolerance. Mycorrhizal fungi are also microorganisms that limit plant stresses and thus indirectly contribute to reduced consumption of agrochemicals (Jamiołkowska *et al.*, 2021). Under salt stress, AMF can improve water and nutrient status, osmotic adjustment, antioxidant defense and hormonal balance of host plants (Ortas et al., 2021). Several studies have reported the beneficial effects of AMF on different citrus rootstocks such as Citrus jambhiri and Poncirus trifoliata, which are widely used for grafting commercial citrus cultivars.(Ortas, 2012) concluded after 3 years of field trials for (seeded) field crops, that natural mycorrhizae make a major contribution to soil and crop management systems to improve plant growth. AMF also increased the contents of soil organic matter, available phosphorus, and hydrolysable nitrogen, but decreased soil EC in saline-alkali soil (Ci *et al.*, 2023). However, further research on the mechanisms and factors involved in AMF-mediated mitigation of salt stress in citrus plants is not fully available. The mechanisms and factors involved in mycorrhizal-mediated salt tolerance of citrus rootstocks are not fully understood and further research is needed. One of the existing studies showed that salt application alone was significantly related to crop stem and root amino acid concentrations, RWC% (Relative water content) and leaf sizes, while mycorrhiza application showed a positive relationship with stem and root wet weight stem height and root amino acids, but led to a decrease in root serine and glutamine and stem amino acid and glutamine (Basak *et al.*, 2019). In another study, it was observed that although plants in the low P + AMF treatment and plants in the high P - AMF treatment had similar P concentrations, mycorrhizal plants had higher dry weight, soluble sugar and electrolyte concentrations in their roots, and similar relationships were observed regardless of the presence or absence of salt stress(Feng *et al.*, 2002). The root-to-shoot ratio of Carrizo citrange (CC) and sour orange (SO) seedlings decreased with mycorrhizal colonization, but the root hydraulic conductivity per unit root length of mycorrhizal Carrizo citrange (CC) and sour orange (SO) seedlings was said to be over twice as high as non-mycorrhizal seedlings under well-watered conditions (Graham and Syvertsen, 1984). Winter rains in Mediterranean climates, particularly in regions with poorly drained soil, can cause flooding stress. Thus, Mediterranean citrus groves may experience annual stressors such as drought, salt, and flooding.

MATERIALS AND METHODS

Site Descriptions and Experimental Setups

In the greenhouse of the Faculty of Agriculture, Soil Science and Plant Nutrition Department at Çukurova University, located in the Eastern part of the Mediterranean region of Turkey a pot experiment was carried out. Saline soil from Gölkaya was used to set up the experiment. The selected soil was collected from the Mediterranean region. The selected soil was divided into 2 parts sterile and non-sterile. 2 different citrus rootstocks [Cleopatra mandarin (Citrus reshni) and C35 citrus] were used in the research. Three different types of mycorrhizal applications such as control, defined indigenous Natural Mycorrhiza and selected *Rhizophagus clarus* were used as mycorrhiza. About 1000 mycorrhizal spores were inoculated to single, healthy, and uniform citrus seedling root surfaces. Each application was replicated three times during the process. There were 36 pots in the trial, and each pot held about 2 kg of soil. The randomized plot design was followed for conducting the trial. The trial was conducted in the Soil Science and Plant Nutrition Research greenhouses of the Faculty of Agriculture, Cukurova University, located in the eastern part of Adana, in the Mediterranean region of Turkey.

Plant stem diameters:

An Electronic LCD Digital Vernier Caliper (Model no. EBC143 China) was used to measure the diameters of the plant stems. The plants' diameter was measured both at the beginning and at 30-day intervals. Throughout the trial, indications of several physiological illnesses, such as leaf necrosis and stem necrosis, were observed in the leaves.

Plant height:

The plants' heights were measured using a measuring tape. The heights of the plants were measured initially and after every 30 days.

Photosystem II (PSII) measurements:

PSII's highest quantum yield was measured each month. Chlorophyll fluorescence efficiency (QY = FV'/FM'; FV' = variable chlorophyll fluorescence value in light-adapted leaf; FM' = maximum chlorophyll fluorescence value in light-adapted leaf) was determined with FluorPenTM fluorometer (Photon System Instruments Ltd, Czech Republic).

Determination of Leaf Chlorophyll Quantity:

The SPAD-502 meter (Minolta, Osaka, Japan) determines the leaf's absorbances in the red and near-infrared spectrums. The meter uses these two absorbances to determine a numerical SPAD value that corresponds to the leaf's chlorophyll content. SPAD readings were taken with an interval of one month.

Statistical Analysis and Data Evaluation

The data was analyzed using a JMP computer program. Tukey's test and Student t test ($p<0.05$) were employed to compare means.

RESULTS AND DISCUSSIONS

SPAD Observations (µmol m^{-2})

SPAD-502 is a simple, portable diagnostic device called a chlorophyll meter which shows how green or how much chlorophyll is present in plants.(Marquard and Tipton, 1987). A popular handheld tool for quickly, accurately, and non-destructively measuring the amounts of chlorophyll in leaves is the SPAD-502 meter. It has been widely used with a variety of plant species for both agricultural and scientific purposes. In the measurements of SPAD, the differences between the mycorrhizal treatments and sterilization status of the soil were non-significant statistically ($p<0.001$), but C35 species performed better than the Cleopatra species as given in Figure 1.

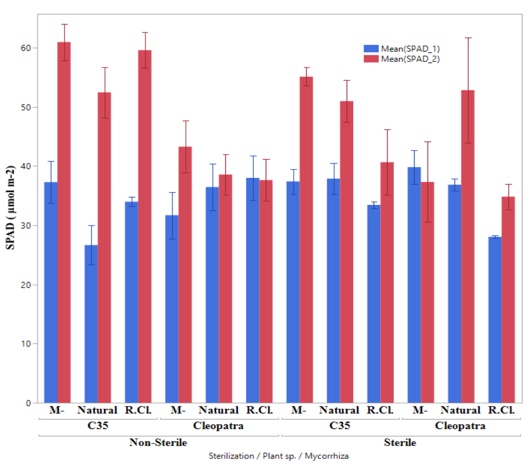

Figure 1. Graph showing the of 1st and 2nd observations of SPAD analysis.

Photosystem II (PS II) Measurements:

According to the study (Jamil *et al.*, 2007), as the salt level rose, the quantum yield of PS II (Fv/Fm), photochemical quenching coefficient (qP), and non-photochemical quenching coefficient (qN) decreased. In this experiment, PS II measurements were taken and results showed that both mycorrhizal treatments were better than the control one statistically ($p<0.001$) also the C35 species performed better than the Cleopatra species as given below in Figure 2.

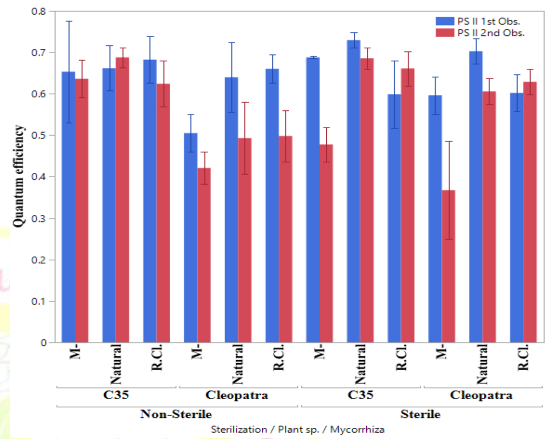

Figure 2. Graph showing 1st and 2nd observations of PS II measurements.

Length Measurement

Vesicular-arbuscular mycorrhizal fungi increase the efficiency of plants in nutrient absorption(Dutra *et al.*, 1996) which causes an increase in plant growth. The results showed that in the first observation, the C35 species performed better than the Cleopatra species with a significant difference statistically ($p<0.001$). All the other factors including mycorrhizae and sterilization were non-significant. In the second observation again C35 species performed better than the Cleopatra species. This difference could be attributed to the physiological differences among the citrus species shown in Figure 3. Also, the non-sterile soil was better than the sterilized soil with a significant difference statistically ($p<0.001$). This difference could be attributed to the fact that in non-sterile soil microorganisms' natural habitat was not disturbed and there were already naturally occurring mycorrhizal spores present in the soil which helped the plant to adapt to the environment.

Diameter Measurements

The results of diameter measurements showed that in both observations C35 species performed better with the highest value of 4.01mm than the Cleopatra species having the highest value of 2.48mm differing significantly ($p<0.001$). All the other factors including mycorrhizae and sterilization were non-significant, which is presented in Figure 4. This difference could be caused due to the physiological differences among the citrus species.

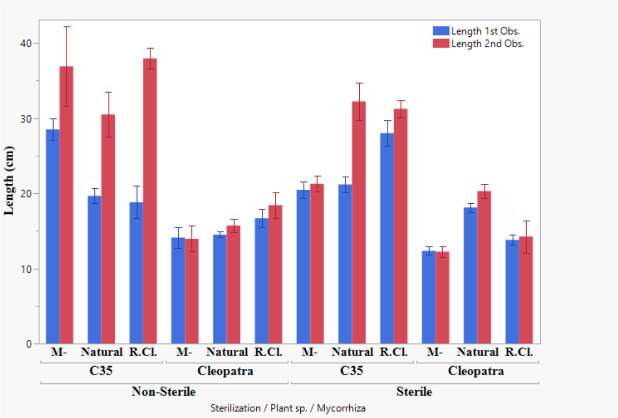

Figure 3. Graph showing 1st and 2nd observations of length measurement

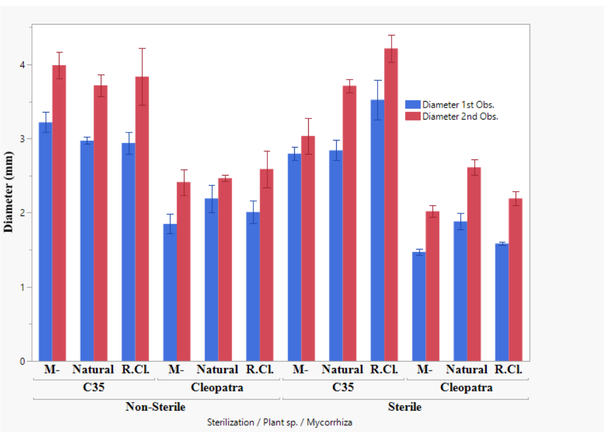

Figure 4. Graph showing 1st and 2nd observations of diameters of plants.

CONCLUSIONS

The results showed that the C35 citrus species are more resistant to natural salt stress than the Cleopatra species. Also, mycorrhizal treatments were more effective in sterilized soil conditions rather than non-sterilized under stress from salinity because of the existence of a natural biome. In the presence of mycorrhizae under saline conditions selected *Rhizophagus clarus* seems to be more efficient than indigenous mycorrhizae. Also, indigenous mycorrhizae significantly affect plant roots under sterile conditions of the soil. It has been seen that mycorrhizae inoculation of the naturally saline-formed soil assisted the plants' seedlings in overcoming salt stress. More growth was observed in the mycorrhizal inoculation treatments in plants exposed to saline soils that had been sterilized. However, there were no notable distinctions between the treatments due to the non-disruption of the biome in non-sterilized soils. To sum up, certain mycorrhiza, particularly native mycorrhiza, can be a good resource to fight environmental challenges like salinity and improve the development of citrus seedlings.

CONFLICT OF INTEREST

The author here declares that there is no conflict of interest in the publication of this article.

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