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# Morphological and Anatomical Development of *Solanum Lycopersicum* Seedlings Grown With Non-Conventional Water

*Research Article*

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## Abstract

Morphological and anatomical measurements of *Solanum lycopersicum* L. seedlings grown with diluted seawater in the greenhouse were analyzed to understand the effects of non-conventional water on the growth and development of the species. The salinity of the non-conventional water ranged from 8.15mS/cm to 9.85mS/cm which corresponds to 0.5% to 2.0% seawater (v/v) in freshwater dilution. The results indicate that no significant difference exists in anatomical and morphological growth and development of the species compared to those grown with freshwater. The study concludes that adoption of this type of non-conventional water resource in greenhouse crop production will save between 415,000 to 1,660,000 liters of freshwater for the United States fresh harvest-producing greenhouses per day. It further concludes that the results represent an effective freshwater conservation strategy for the United States and the world at large.

**Keywords:** Non-Conventional Water, Seawater, Freshwater, Water Conservation, Greenhouse

**Abbreviations:** EC: Soil Electrical Conductivity; CC: chlorophyll content; LT: Leaf Temperature; CWT: Cell wall Thickness; LD: Lumen Diameter; ET: Epidermal Thickness; PML: Palisade Mesophyll Length; PMW: Width of Palisade Mesophyll; RCBD: Randomized Complete Block Design; RGR: Compute Relative Growth Rate: RT: Rate of Transpiration; LA: Leaf area

## Introduction

One of the world's most in-demand natural resources is freshwater. Agriculture, the greatest single user of freshwater, uses approximately 75% of the Earth's freshwater. Agricultural use, in some parts of the world, can be up to 90%. These usage amounts, of a natural resource whose availability is only 3% of the earth's water resource, by a single human socio-economic sector is alarming [1,2]. There are about 463,365 ha of fresh harvest-producing greenhouses in the United States [3], which use about 83,000,000 liters of fresh water every day [4]. At this rate of usage, groundwater and/or freshwater resource will deplete sooner especially when the bourgeoning human population is factored into freshwater consumption. To meet urban, industrial and other human needs of freshwater demands, water must be diverted away from agricultural irrigation. This diversion away from agriculture, however, will impact human food security if alternative or non-conventional water sources are not developed to meet agricultural crop production. The scarcity of freshwater supplies has become increasingly acute, and it is a growing problem in most developing countries. As a result, alternate means of subsidizing the burden on freshwater utilization is required, and this will involve utilization of non-conventional or non-traditional water sources. Although when the salinity of irrigation water exceeds the tolerance level of a species, the decline in yield of that species is unavoidable. Because of this, it was suggested that saline water irrigation at lower concentrations could be quite effective as an alternative agricultural water source and for preserving freshwater [4-6].

Tomato has gained much popularity in the United States over the past 50 years. It is widely cultivated across the world because of its pleasant taste, variety of culinary uses and nutrient content including vitamin C, potassium, and carotenoids [7]. Global output of tomatoes, including tomato crops grown under saline water conditions, have climbed by more than 54% between 2000 and 2017 [8]. Romero-Aranda et al. [9] noted that tomato crops grown with saline water transpired less compared to crops grown with freshwater. Could this be advantageous to the burgeoning human population to save freshwater if yield is not compromised by growing tomato with saline water? Our objective is to evaluate growth, development and yield of tomato grown with diluted seawater as a conservation strategy of freshwater in sustainable greenhouse operation.

## Materials and Methods

*Solanum Lycopersicum* (Roma tomato) seedlings were grown from seed in the Department of Agriculture, Agribusiness & Environmental Sciences greenhouse located at Latitude 27°31'50.3" N and Longitude 97 ° 53'13.7" W. Roma tomato seeds were broadcast in shallow trays containing media mix of 2:1:1:1 topsoil, vermiculite, peat moss and perlite respectively. Germination occurred in less than fourteen days, and germinated seedlings were transplanted into celled flats and allowed to grow for 16 days before further transplantation into 20cm diameter pots filled with the same potting mixture. A total of 175 seedlings were transplanted and arranged in a randomized complete block design (RCBD) of five treatments, replicated in five blocks with each block containing seven plants of each treatment.

The plants were irrigated with different concentrations of saline water according to the treatment. Saline water was obtained from seawater collected from Baffin Bay, Texas and diluted with the City of Kingsville, TX freshwater in the following proportions: 6.86mS/cm (Control with no seawater), 8.15mS/cm (0.5% seawater v/v), 8.94mS/cm (1.0%), 9.43mS/cm (1.5%), and 9.85mS/cm (2.0%). These dilutions are below the threshold identified previously [10-12] to cause physiological and morphological reduction and/or distortion in the species growth and development. Each plant was watered with 1,000 ml of the diluted seawater weekly according to the treatment groups, and morphological and physiological parameter measurements were recorded every 14 days.

The growth, development and environmental parameters measured include chlorophyll content (SPAD units), height (cm) was used to compute relative growth rate (RGR), soil electrical conductivity (mS/cm), leaf temperature (°C), rate of transpiration (ml/h) and leaf area ($cm^2$) Chlorophyll content (CC) was measured with the SPAD-502 Plus chlorophyll meter manufactured by Konica Minolta, Japan. Soil electrical conductivity (EC) was measured with the Hanna Instruments Portable Water Conductivity & Soil Activity Meter while leaf temperature (LT) was measured with the Cole Parmer Infrared Thermometer. The gravimetric method also known as weight-loss method was used to determine the rate of transpiration (RT) of the plants under the different salinity irrigation treatments. Leaf area (LA) was measured with the CI-202 Portable Laser Leaf Area meter manufactured by CID Bio-Science, Washington, USA. Twenty randomly selected fruits from each treatment were squeezed off water, dried at 90°C and weighed every seven days until reaching a constant dry weight. The fruits were individually weighed and the dry weights were subjected to t-test analysis by treatment. Cell wall thickness (CWT), lumen diameter (LD),Epidermal thickness (ET), palisade mesophyll length (PML) and width of palisade mesophyll (PMW) of plants irrigated with the different saline water concentrations were measured to understand the effects of the treatments on the anatomical development of the species. Randomly selected mature leaves from each treatment were fixed in FAA (Formalin-Acetic Acid-Alcohol) and moved to the laboratory for processing. The fixative, 50ml alcohol (95%), 5 ml glacial acetic acid, 10ml formaldehyde, and 35ml distilled water, was used to stop all physiological processes as the samples were transported to the lab. Further laboratory processing of the samples including dehydration, embedding in wax, sectioning, mounting on slides for microscopic measurement, hydration, and staining were according to Berlyn and Miksche [13]. The specimens were sectioned at 6µm for anatomical measurement with a calibrated stage micrometer. Lumen diameter was recorded as the average of the longitudinal measurement taken at 12–6 o' clock direction, and a corresponding measurement of the same lumen measured in the latitudinal direction at 9–3 o' clock direction perpendicular to the longitudinal measurement (Figure 1). All data was statistically analyzed using SAS 9.01.

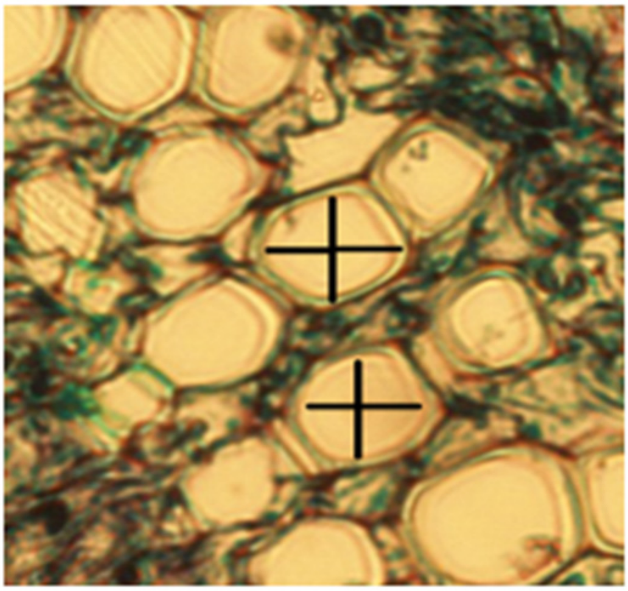

**Figure 1:** Leaf cell wall and lumen sections measured.

## Results

The results show that EC of the blended water used in irrigation was significantly reduced by the potting mixture (Figure 2) although the potting mixture EC increased with increase in seawater EC. The reduction in EC by the potting mixture is significantly different between the treatments and the control (Table 1). However, except for the lowest seawater-freshwater blend, no significant difference in EC exists between the other treatments. ANOVA statistic applied to decipher the effects of seawater-freshwater blend used in irrigation on the morphological and anatomical growth and development of the species indicates that the increase in potting mixture EC due to increase in the amount of seawater in the dilution has no significant effect on the morphological and anatomical development of S. *Lycopersicum* (Table 1).

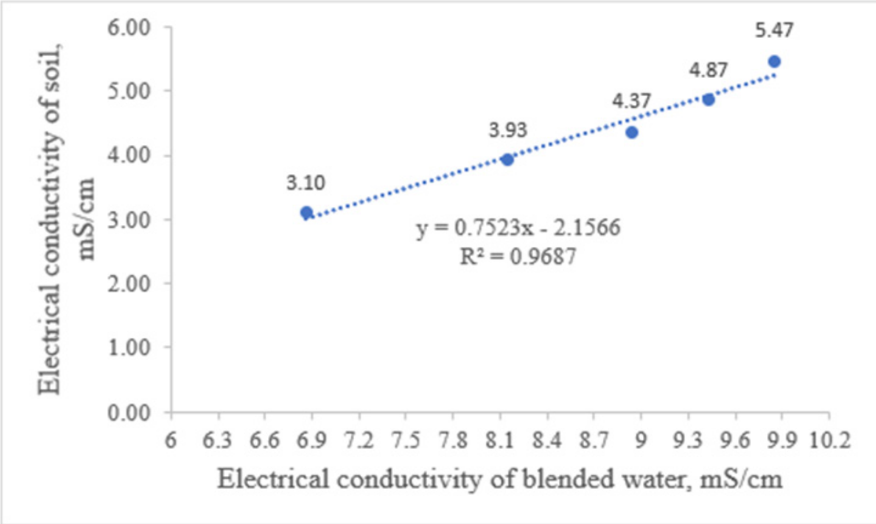


**Figure 2**: Effect of blended seawater on electrical conductivity of potting mixture.

**Table 1:** Effects of seawater-freshwater blend on S. *Lycopersicum* morphological and anatomical development, and potting mixture.

| | **6.86 mS/cm** | **8.15 mS/cm** | **8.94 mS/cm** | **9.43 mS/cm** | **9.85 mS/cm** | **P-value** |
|---|---|---|---|---|---|---|
| RGR cm/day | 8.610[a] | 8.173[a] | 8.257[a] | 8.162[a] | 7.448[a] | 0.4295 |
| Soil EC | 3.101[a] | 3.934[b] | 4.365[c] | 4.869[c] | 5.471[c] | <0.0001 |
| RT (ml/h) | 2.1168[a] | 2.457[a] | 1.9404[a] | 1.9656[a] | 2.326[a] | 0.6455 |
| LA ($cm^2$) | 1519.475[a] | 1453.676[a] | 1544.378[a] | 1575.122[a] | 1474.09[a] | 0.3799 |
| CC (SPAD Units) | 25.68[a] | 25.94[a] | 24.11 [a] | 26.15[a] | 26.11[a] | 0.2568 |
| LT ($^0$C) | 18.68[a] | 16.84[a] | 16.74[a] | 16.69[a] | 18.14[a] | 0.0631 |
| Yield (g) | 178.58[a] | 168.6[a] | 184.4[a] | 175.6[a] | 192.06[a] | 0.3377 |
| ET (mm) | 0.02[a] | 0.02[a] | 0.02[a] | 0.02[a] | 0.02[a] | >0.9975 |
| CWT (mm) | 0.02[a] | 0.02[a] | 0.02[a] | 0.02[a] | 0.02[a] | >0.9974 |
| LD (mm) | 0.01[a] | 0.01[a] | 0.01[a] | 0.01[a] | 0.01[a] | >0.9999 |
| PML (mm) | 0.072[a] | 0.077[a] | 0.073[a] | 0.073[a] | 0.071[a] | >0.9999 |
| PMW (mm) | 0.040[a] | 0.377[a] | 0.037[a] | 0.037[a] | 0.037[a] | >0.9784 |

**Note**: Numbers with the same letter superscript are not statistically significantly different. All numbers are means of each treatment.

## Discussion

Plant species, including agricultural crops, are sessile organisms that rely on soil water for survival. Survival and optimum yield of agricultural crops guarantee human food security. Unfortunately, freshwater on which plants rely for optimum growth and development has become a rare natural resource and threatens food security if the competition between agriculture and humans for its use is not reduced or eliminated. Therefore, it is imperative that alternative water sources are developed. The current research evaluates growth and development of tomato in greenhouse conditions by using diluted seawater. The study adopted a real-life scenario in which its results can be easily adopted in field conditions by using the most abundant water resource on earth. The study did not irrigate with dissolved NaCl in freshwater to simulate a saline soil environment, rather we used natural seawater mixed with a local municipal water source. Also, the study adopted a method that can be applied easily in the gulf coast region of the United States, where the fresh harvest-producing greenhouses are in proximity of coastal seawaters.

It has been documented that the 463,365 ha of fresh harvest-producing greenhouses in the United States use about 83,000,000 liters of fresh water per day [3]. Even a 1% seawater dilution with freshwater used in greenhouse crop irrigation will save 830,000 liters of freshwater every day in the United States alone, and a 2% dilution as in this study will save 1,660,000 liters per day. By extension, this will save billions of liters of freshwater world-wide every day. It is important to stress that the results of the study apply to greenhouse conditions only as it has been widely documented that open field flood irrigation and the associated evapotranspiration cause salt build-up [14,15]. The reduction in potting mixture EC in this study is significant and further confirms the buffering capacity of soil. This has implication in the field environment if potted plants grown with diluted seawater are planted in the field. The larger soil condition of the ambient environment will resist any change in EC of the field soil environment.

An important factor that deserves special attention is the seawater-freshwater dilution and the resultant EC of the mixture. This makes the use percent seawater in the dilution unreliable. For example, a 2% (v/v) of Baffin Bay seawater and Kingsville freshwater resulted to 9.85mS/cm. This may not be the case with seawater from another location diluted with freshwater from a different city. Therefore, the EC as measured in Siemens unit is a better measure than percent quantity. Also, the sensitivity of crops to salinity has been documented in several reports [16-18]. The salinity of any medium is, in part, due to the soil. We used a potting mixture of 2:1:1:1 topsoil, vermiculite, peat moss, perlite which significantly lowered the EC of the potting mixture relative to the EC of the blended water. Therefore, a different potting mixture will react differently with the EC of the diluted seawater. It is therefore important to know the EC of the seawater-freshwater blend of your location before the mixture is used in greenhouse crop irrigation.

Our results indicate no compromised physiological or morphological parameter of the plant species due to the salinity treatments. It will be wrong to assume that the situation will be the same for all greenhouse crop species. We advise that each crop species be evaluated for saline tolerance threshold before wide application of blended water in greenhouse crop production. Also, it is necessary to evaluate all agricultural crop species grown in large quantities in greenhouses globally with a focus of developing a saline tolerance threshold atlas for each species. Such a novel development will serve as world-wide conservation blueprint for greenhouse growers in freshwater resource conservation.

## Conclusion

We conclude, based on the evidence in this greenhouse study, that Roma tomato can be grown with diluted seawater without compromising yield. The range of EC of the diluted seawater must not exceed 9.85mS/cm for the variety. Seawater-freshwater blend must not be based on v/v percentages alone without confirming the EC of the mixture before greenhouse irrigation.

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## Conflict of Interest

The authors attest to no conflicting interest to the content of this manuscript or part of it. The authors further attest that the manuscript is not submitted to any other journal.

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