Determining the Most Effective Salt Concentration of Irrigation Water for *Trichoderma harzianum* to Confer Salt Tolerance Through Symbiosis to *Oryza sativa* Plants

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Abstract

As Earth's climate continues to rapidly change, rising sea levels and pressure on fresh water reserves have made many staple crop plants, especially rice, extremely vulnerable. With arable coastal land quickly becoming salty and unconducive to plant growth, it is more important now than ever to discover new ways to grow important crops in harsher environments. This study sought to find how different concentrations of salt in irrigation water affect rice plants in symbiotic relationships with the *Trichoderma harzianum* fungi species versus rice plants by themselves. Rice plants with and without symbiotic relationships with the fungi species were grown for six weeks and irrigated with a range of solutions from fresh water to 300 mM salt water. The height, color, and wiltage level of each plant were measured as indication of its healthiness. The data collected showed with statistical significance that the plants grown in conjugation with the fungi and irrigated with a 300 mM salt water solution were the healthiest in terms of their growth rate, color and wiltage level, with a p-value of 0.002. This study showed the most efficient ways of utilizing fungal symbiosis in rice plants and taking advantage of the increased salt levels in coastal soils to generate healthier crops. These results can be applied to improve crop growth in the salt-degraded areas which compose 20 percent of the world's irrigated land.

Introduction

Across the globe, more than 700 million people still remain undernourished. Much of this hunger is due to agricultural issues in the face of long-term climate risks. Increasingly, seawater has encroached into farmland in coastal regions and compromised its fertility, placing stress on the crops and threatening the productivity of the farmland. Research has shown that in habitats of high environmental stress, symbiosis between a host plant and its endophytic fungi allows for stress tolerance to confer from the fungi to the plant, leading to higher rates of plant survival (Rodriguez et al., 2004). Previous studies have discovered the ability of such endophytes to be removed from their native host plant and added to a new plant with success in improving the new host's tolerance, as well as how such a transfer would improve crop strength in climate-change-afflicted areas. (Rodriguez et al., 2008). This experiment seeks to investigate a range of saline concentrations to determine what level of salt tolerance may be conferred to Asian rice (*Oryza sativa*) plants when grown symbiotically with the *Trichoderma harzianum* fungus. *T. harzianum* is a cosmopolitan fungus found through the world in soils and associated with plants and has been shown to confer various stress abiotic tolerances such as drought and temperature tolerance, and promote growth enhancement. However, the ability of *T. harzianum* to confer salt tolerance has not been as well studied as in other symbiotic systems such as the symbiotic association of the *Fusarium culmorum* fungus with host plant *Leymus mollis* (a species of dunegrass),whose natural habitat is in salty coastal waters. Studies have shown that this fungus is able to symbiotically confer very high levels of salt stress tolerance (up to sea water levels) to its host plant as well as other plant species (Redman et al., 2011), but is not commercially available for use. Since *T. harzianum* is available commercially and is a non-pathogenic species, it was of interest to see if a high level of salt tolerance could be imparted using this system. *Oryza sativa* and *Leymus mollis* are both grasses, are genetically similar, the symbiont used in this study known to have a broad plant host range, all of which indicate that salt tolerance may be conferred symbiotically to rice. The objective of this experiment is to test a range of salt water irrigation concentrations to determine if salt tolerance can be achieved, and to which concentrations will lead to the most productive and healthy *Oryza sativa* plant in symbiosis with *Trichoderma harzianum*, in terms of height, color, and wiltage levels.

Methodology

One hundred and twenty seedlings were first surface sterilized and then germinated for one week. Half of the viable seedlings were treated with the fungal solution, while the other half were treated with distilled water for 24 hours.

A triple-decker planter box system (Figure 1) was used in this study, allocating separate sections for water (lower chamber), soil (middle chamber), and plant growth (upper chamber). A wicking system allowed the water, salt, and nutrients in the bottom box to be drawn naturally upwards, hydrating the soil matrix in the middle chamber. A sponge was placed in the top lid to promote carbon dioxide and oxygen exchange without risk of contamination, since the contaminants were able to move throughout the air. The wicking system was constructed from cotton rope, and the soil received an initial hydration with distilled water. All of the boxes were sterilized before transplantation.

In order to best control what entered the experimental environment, a fertilizer solution was made to mimic the effects of a standard commercial fertilizer which a farmer in the affected region may use. $CaCl_2$, NPK, and Fe were combined with distilled water (in a 1:1:1 ratio), and added in equal amounts to each planter box. NaCl was added in the same concentration to distilled water to make a salt stock solution.

Four rice seedlings were transplanted randomly into each planter box and kept for 12 hours each day under fluorescent light. Control and experimental plants were kept in separate boxes to prevent cross contamination of the fungi. Following one initial week of growth, the lower chamber of the experimental plant boxes were filled with the appropriate salt water concentration, rather than the sterile water. Each week for five weeks, the heights of all the plants (cm), the wiltage level, and the color of the leaves, were measured and recorded. Leaf wilt levels and color retention were measured on a subjective scale from one to five. The amount of wilting and coloration of leaves are indicators of stress, with high stressed plants showing severe wilting and loss of green coloration in the leaves (chlorosis). For wiltage, a scale level of one represented a dead plant, three represented a moderately wilted plant with low levels of chlorosis, and five represented a fully upright plant. For color, one represented high chlorosis with pale-yellow green leaves (indicative of plant death), three represented medium green colored leaves indicating a moderate level of chlorosis, and five represented deep green leaves with no chlorosis.

The treatments were designed such that each triple decker magenta box contained four plants symbiotically colonized with the fungus and referred to as experimental, or four control plants without the fungus and referred to as control. In total, 48 symbiotic (experimental) and 48 non symbiotic (control) plants were assayed for the entire study. The experiment was independently repeated three times. Each trial was organized as shown in Figure 2.

Figure 1: Planter Setup

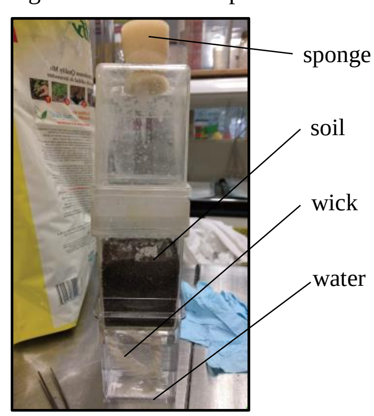

Figure 2: Experimental Setup

experimental	experimental	experimental	experimental	Plants Inoculated with Fungi
control	control	control	control	Plants not Inoculated with Fungi
0 M	100 mM	200 mM	300 mM	

NaCl Molarity of Irrigation Water

Results and Analysis

Figure 3 (below): This graph shows the growth progression of each experimental group.

The growth rate of the 300 mM symbiotic plants was determined to be the highest, followed by the 100 mM non-symbiotic plants. The 300 mM symbiotic plants were the tallest at week 0 and remained the tallest for the duration of the experiment. This may correlate to the fungal presence's impact on the overall health of the plants (Redman, 2018). The percentage changes in height of the symbiotic plant groups were greater than those of the non-symbiotic groups, with the greatest height change occurring in the symbiotic 200 mM group

Figure 4 (below): This graph shows the changes in color of the experimental groups over the five-week period.

Using a subjective visual scale (from 1-5), it was found that most groups began with deep green color, indicative of full health, and represented by a value of 5. The 300 mM symbiotic group retained the highest color value for the duration of the experiment. The 200 and 300 mM non-symbiotic plants had the lowest color values by week 2; and this trend continued through week 4.

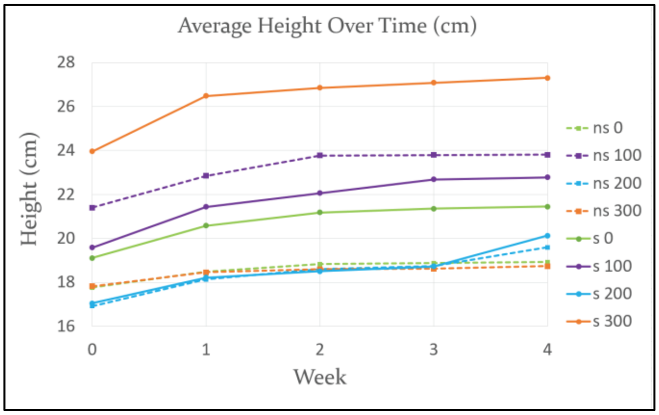

Figure 3

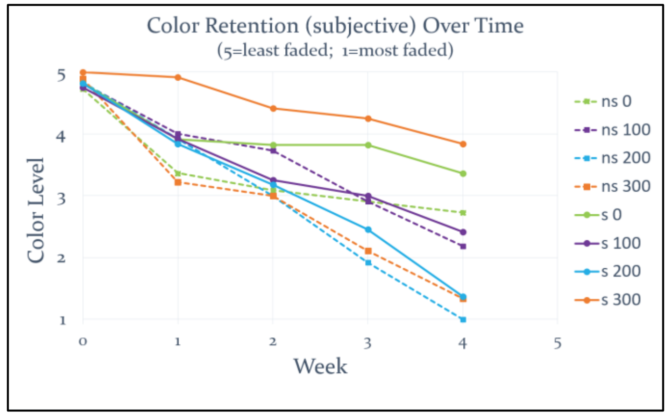

Figure 4

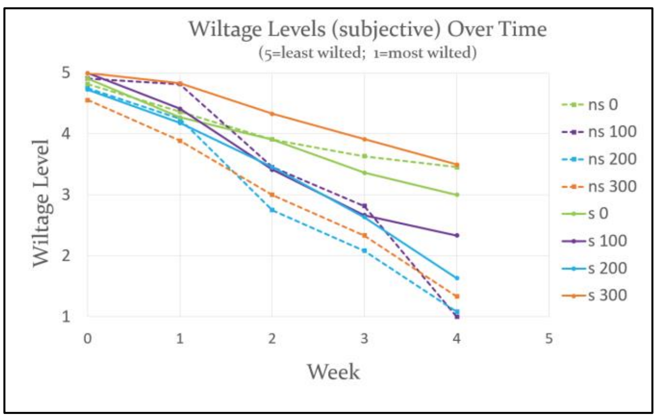

Figure 5

Figure 5 (left): This graph shows the change in average wiltage level over time.

To determine the plants' wiltage levels quantitively, a subjective visual scale was developed (from 1-5). Figure 4 shows that most groups had a value of 5 at the start of the experiment, which is representative of completely upright, fully healthy plants. The 300 mM symbiotic plants experienced a steady decline in wiltage values, but remained the most upright throughout the five weeks. The 200 and 300 mM non-symbiotic plants were consistently the most wilted, and ended the experiment with values of 1, which is indicative of plant death.

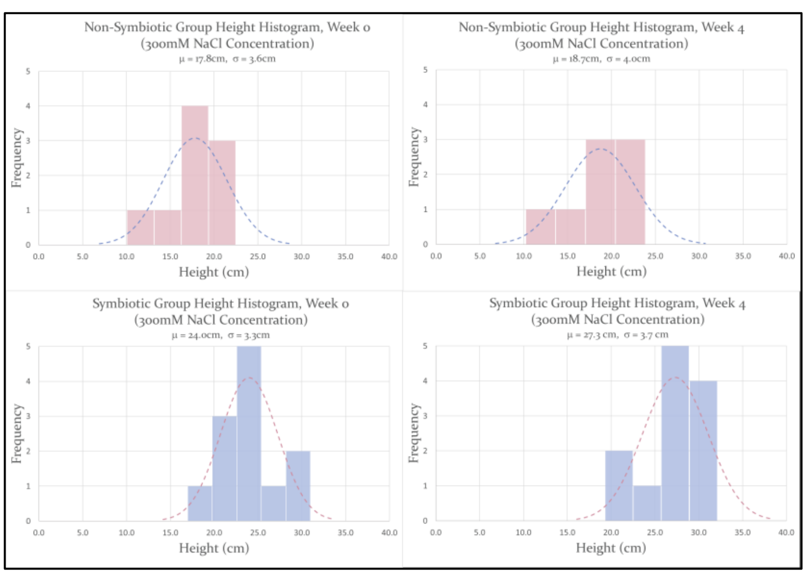

Figure 6

Figure 6 (left): These histograms and normal curves show the height distributions of the symbiotic and non-symbiotic plants irrigated with 300 mM water over the course of the study. The non-symbiotic plant groups experienced an almost negligible shift in height distribution between the beginning and end, which suggests that the plant growth was inhibited by the NaCl in the water. The symbiotic plants experienced a significant positive shift in height over the course of the study which suggests that the fungi plant relationship was successful in mitigating the stress of a saline environment. In addition, the 0 mM symbiotic plants were found to be taller and greener than their non-symbiotic counterparts, indicating that the simple establishment of the symbiosis between plant and fungus provided positive benefits, even in the absence of stress.

Conclusion

This experiment sought to find how different concentrations of salt in irrigation water affect rice plants in symbiotic relationships with the *Trichoderma harzianum* fungi species versus rice plants by themselves. Experimental methods were carefully implemented to ensure that the nutrient and salt concentrations were regulated, and that the risk of contamination between experimental groups was minimized.

The results demonstrated that the plant groups grown in conjugation with the symbiotic fungi had higher growth rates, lesser wiltage levels and greater color retention than plants lacking the symbiotic association when exposed to a salt stresses. The differences between these factors for symbiotic and non-symbiotic plants irrigated with 300 mM salt water was found to be statistically significant using analysis with a t-test. P-values of 0.002 (growth rate), 0.00001 (wiltage) and 0.00006 (color) were derived, which mean that the null hypothesis can be rejected. These results also indicate that the hypothesis that this symbiotic experimental group would be the most successful in terms of plant health and survival was confirmed.

Future experimentation could include replication of this study in an environment in which *Oryza sativa* is traditionally grown, in order to see how the introduction of *Trichoderma harzianum*, a non-native species, would impact its new ecosystem. Such a field study would also provide insight into how this solution would fare in a less-controlled environment, and if it is reliable enough to become widely used. Extending the range of salt water concentrations tested would also be of value for determining the viability of the rice plant growth in areas where the salinity of ocean water in higher than average.

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Citations

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