Morphological Response of Radishes Foliarly Treated with Chitosan in Saline Soil

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Abstract

High salinity soil creates osmotic pressure that causes stress in plants, inhibiting nutrient uptake, and killing vegetation. Chitosan, a natural bio-stimulant that is known to reduce abiotic stress, can increase nutrient uptake, and accelerate growth. The purpose of the experiment was to find the optimal dosage of chitosan that correlates to the most morphological growth in radish plants in high saline soil. Radish seeds were soaked in 0.01%, 0.05%, 0.1%, and 1% chitosan solutions and planted in high salinity soil. Once sprouted the following week, plants were given a weekly foliar application of their respective chitosan solution until harvested for a total of four weeks. All the chitosan radishes produced larger leaf areas and dry biomass weight compared to the control group not treated with chitosan, with the 0.01% solution producing the largest leaf areas and the 1% solution yielding the highest dry biomass.

Introduction

With the ever-present threat of global warming, sea levels are predicted to rise by 12 inches by the year 2050.[1] With this rising, salt water will be even more present and available to be absorbed by fertile soil, rendering it saline and unusable for the agricultural industry. It is estimated that 150,000 km^2 are located in low-elevation coastal zones meaning that they are at risk for saline contamination as sea levels rise.[2] Even now, soil with high levels of salt is a major global issue plaguing the agriculture industry. Roughly 1.38 billion hectares of soil is classified as saline. This threatens the food security of roughly 1.5 billion people.[3] Chitosan, a natural biopolymer derived from the chitin shells of crustaceans and some fungi discovered in 1859 that has received interest recently, has begun to be used to combat this issue as it has been shown to increase the resilience of plants in poor soil conditions. When grown in these saline environments, the application of chitosan in seed dressing as well as foliar application to the shoot system can reduce the oxidative stress by boosting a plant's antioxidant defense system, lowers reactive oxygen species accumulation, and improves ionic homeostasis and increase the moisture retention. While chitosan has been shown to be beneficial for plants in saline soil, the goldilocks dosage that should be used is still up for discussion. Current research shows mixed data on morphological growth in plants treated with chitosan including both positive and negative effects across dose ranges.[4,5] This experiment sought to find the optimal foliar dosage or dosing range of chitosan for Champion Radishes to promote optimal levels of morphological growth. It was hypothesized that a lower chitosan solution of 0.1% used in the foliar application to Champion Radishes will produce the greatest morphological growth in high saline treated soil.

Methods

Materials

- 1% chitosan stock solution (pH ~6.6)
- Diluted chitosan solutions (0.01%, 0.05%, 0.1%)
- 2 Thermo Scientific Nalgene 34 oz bottles
- 4 VWR 250 mL media bottles
- 3 Kiloline Pro PET plastic 200 mL spray bottles
- Ferry-Morse champion radish seeds
- Soil provided by WCC lab techs
- 6.4 g/L NaCl solution
- 10 plants pots (6 in. diameter)
- Graduated cylinders (10 mL, 50 mL, 100 mL)
- Lithonia Lighting Fluorescent Fixture Suitable For Damp Locations (0.75 Amps, 120 Volts, 60 Hz)

Procedure

1. Add 350 g of soil to each of the 10 pots labeled with proper chitosan concentrations (control, 0.01%, 0.05%, 0.1%, 1%)
2. Add 6.4 g of NaCl to 1 L of dH2O to create saline solution
3. Create diluted chitosan solutions from 1% chitosan stock solution
    a. 90 mL dH2O + 10 mL 1% stock solution = 100 mL 0.1% chitosan solution
    b. 95 mL dH2O + 5 mL 1% stock solution = 100 mL 0.05% chitosan solution
    c. 99 mL dH2O + 1 mL 1% stock solution = 100 mL 0.01% chitosan solution
4. Add 200 mL of 6.4 g/L NaCl solution to each pot to soak soil
5. Spray seeds 3 times with respective chitosan concentrations & let sit for 30 minutes
6. Plant 4 radish seeds 1/2 deep in each pot, ~2 inches apart
7. Water soil of each pot with 100 mL of water every 3-4 days
8. Apply 3 sprays of the proper chitosan solution to each pot every Tuesday for 4 weeks once seeds begin to sprout
9. After 23 days, take measurements for one pot of each concentration
    a. Plant Height: Measure from soil to first stem branch point
    b. Leaf Area: Measure length & width of largest heart from each plant and multiply by correction factor of 0.75. Divide area by number of plants, then multiply by the total number of leaves in the pot.
    c. Biomass: Place in incubation chamber at 70 °C for 24 hours to dehydrate
10. After 28 days, repeat measurements for the remaining pot of each concentration

Morphological Growth Parameters of Radishes After Four Weeks of Chitosan Treatment

	Control 4 Weeks	0.01% 4 Weeks	0.05% 4 Weeks	0.1% 4 Weeks	1% 4 Weeks
Plant Height (cm)	5.833	6.625	5.78	6.125	7.075
Leaf Area (cm^2)	57.09	86.7	76.68	79.92	82.21
Dry Weight (mg)	117.8	215.6	222.3	213.3	236.7

Table 1. Results of radish growth measured by plant height (cm) from soil to first stem branch, total leaf area (cm^2) calculated as length × width × a correction factor of 0.75, and dry weight (mg) after 24 hours in an incubation chamber at 70 ºC.

Effects of Chitosan Concentrations on Radish Leaf Area and Dry Weight

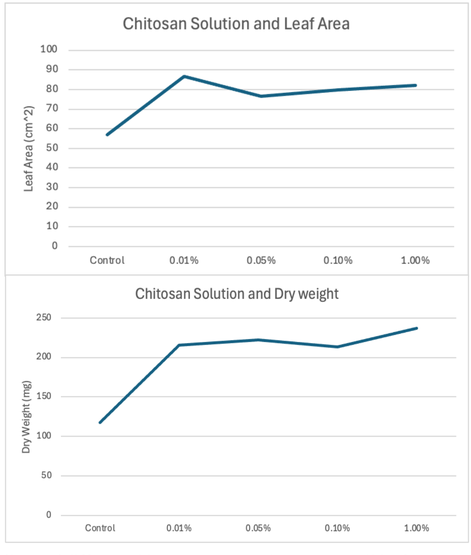

Figure 2a. Effects of different chitosan concentrations on overall leaf area (cm^2) in radishes. Leaf area was lowest in control, reached its maximum at 0.01%, decreased at 0.05%, then slightly increased at higher concentrations.

Figure 2b. Effects of different chitosan concentrations on total dry weight (mg) in radishes. Dry weight was lowest in control, increased at 0.01%, remained stable across intermediate concentrations, and reached its maximum at 1%.

Discussion

After running the experiment of foliar application of chitosan to Champion Radishes in saline soil for four weeks, the hypothesis that the radishes receiving 0.1% chitosan would yield the highest morphological growth markers was found to be incorrect. The control group, radishes in saline soil that did not receive foliarly applied chitosan, performed the worst across every category of morphological growth. In comparison, the radishes that received foliar application of a 1% chitosan showed the highest amount of morphological growth in dry weight and height but slightly less leaf area than the 0.01% radishes. The 0.1% chitosan solution showed great growth compared to the control, but marginal morphological growth compared to the other chitosan solutions. The 0.01% chitosan solution produced plants with the largest overall leaf area as well as performing solidly in the other two measured categories. Because of this, it was determined that the 0.01% solution that led to the largest leaf area, was the optimal dosage as it would allow for the greatest opportunity for photosynthesis, promoting radish bulb growth through the opportunity for increased glucose production.

The efficiency of lower levels of chitosan was seen in a 2016 study in the Pakistan Journal of Agricultural Research where the highest and most beneficial levels of morphological growth in summer tomatoes were seen in the chitosan levels ranging from 0.0075% to 0.01%.[5] While the results of the research conducted in this study aligns with previous research, the original hypothesis that the 0.1% chitosan would yield the best morphological growth was determined because of the variable of growing in poor soil conditions. The higher strength solution was hypothesized to counter the poor soil but was determined to not lead to more growth than lower levels. With the lower levels of morphological growth of the 0.05% and 0.1% solution, it was determined that these levels of chitosan may have acted as a bio-inhibitor rather than stimulant. As for the growth seen in the 1% chitosan solution, it is believed that since chitosan is known to trigger the defense mechanisms of plants,[4] the 1% solution could have triggered more primary and secondary growth in the radish leading to thicker and longer stems with less leaf growth than seen in the 0.01% solution.

While this study yielded no radish bulbs, this is believed to be from the time constraint of this experiment. Data from radish plants harvested five days prior to the final harvesting showed much lower levels of morphological growth with disparities of almost 80 mg of dry weight gained in those five days in the 0.01% solution and 20 cm^2 of leaf area gained by the 1% solution. This rapid vegetative growth is believed to be due to the radishes attempting to maximize their energy gathering ability as the radish bulb was beginning to grow.

Future research could be done on the effects of chitosan in other poor soil conditions, as well as with other crop types to maximize production using chitosan. Globally, as soil conditions become more saline and no longer available for the use of farming, it is incredibly important to find a way to keep up with the ever-growing demand for food. Chitosan could be the bridge to healthy communities worldwide.

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