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Stress Response to Different Concentrations of NaCl: Analysis of Root Length and Protein Expression on Wild Type *Arabidopsis thaliana*

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Abstract

The purpose of this experiment was to examine the stress response of wild type *Arabidopsis thaliana* to treatment with different sodium chloride concentrations. Previous research has shown that sodium chloride induces a stress response from plants in both a physical sense and molecular sense. The hypothesis was that the plants would exhibit a number of different stress responses including shorter root length and an increased production in specific stress- responsive proteins. The hypothesis was tested by placing germinated *Arabidopsis* seedlings onto MS (Murashige and Skoog) agar plates with varying sodium chloride concentrations. While the seedlings grew on the plates with sodium chloride, root length was measured. After root length measurements were completed, specific plants from each concentration were subjected to protein analysis through SDS-PAGE to compare the banding patterns of protein expression for each treatment group versus the control. The importance of this study was to better understand how plants tolerate different levels of sodium chloride. Root length analysis showed that higher concentrations of sodium chloride dramatically inhibited a plant's growth. SDS-PAGE analysis showed a protein band that was present in the control plants and was not present in the plants that grew in an added concentration of sodium chloride. These results show that the plants have specific stress responses to sodium chloride including shorter root length and a decrease in the production of an unkown protein.

Introduction

Roots are the primary site in plants for water- related stress. Since the roots are where the main source of water absorption takes place, it would make sense that it is the site where water-limiting stress would occur. The problem with this is that the roots help to control the productivity of the entire plant, and when the roots don't function properly, the rest of the plant suffers because of it[1]. Organisms will often express proteins in response to stress. Sodium chloride, or NaCl, has been shown to induce specific stress responses including a decrease in the plant's productivity and an increase in the production of certain stress related proteins in Arabidopsis. Based on the research of previous scientists, an interesting question to study is whether different concentrations of NaCl inflict different stress responses on *Arabidopsis thaliana*?

In a study by Deyholos and Jiang, the scientists wanted to further understand the plants responses to abiotic stress by completing a microarray analysis on the Arabidopsis roots after an exposure to NaCl[2]. The Arabidopsis plants were grown hydroponically, and after 21 days of germination, they were given a 150mM supplement of NaCl. After analyzing the results, they identified different transcriptional responses in the plant, which indicated the production of new proteins, most likely stress-responsive[1]. By completing this experiment, the hope was to gain further knowledge on salt tolerance in plants to try and possibly improve salt tolerance in other plants. The experiment question stated above would build off of this research by further testing to see how NaCl affects the rest of the plant's productivity.

In another study by Jiang and other researchers, the scientists were continuing their earlier studies described above, focusing on the production of specific stress-responsive proteins. The purpose was to better understand the proteomic level of NaCl stress responses in *Arabidopsis* roots[1]. After completing a procedure similar to the one above, instead of completing a microarray analysis, the scientists performed electrophoresis to separate and analyze the production of specific proteins that are commonly stress-responsive. They found that post-transcriptional gene regulation plays an important part in expressing the stress-responsive proteins. This experiment would relate to the proposed research question above because they used electrophoresis to perform a proteomic analysis of stress-responsive protein production in NaCl treated *Arabidopsis* roots[1].

In testing how different concentrations of NaCl inflict different stress responses on *Arabidopsis thaliana*, it is hypothesized that the plants will exhibit a number of different stress responses including shorter root length and an increased production in specific stress-responsive proteins. This hypothesis will be tested by placing germinated *Arabidopsis* seedlings onto agar plates with different NaCl concentrations. While the seedlings grow on the plates with NaCl, root length will be measured. After allowing the seedlings to grow for a certain amount of time on the agar, a certain number of plants from each concentration will be removed to test for a protein analysis through a SDS-PAGE to compare banding patterns for different concentrations with the control. The importance of this study is to better understand how plants tolerate different levels of NaCl in an attempt to improve salt tolerance in other plants. If we understand how *Arabidopsis* uses certain proteins to deal with tolerating different levels of salts, those proteins could possibly be isolated and used in other plants to help them deal with salt tolerance too. It is important to understand salt tolerance in *Arabidopsis* so that we can expand our ability to select and engineer better salt tolerant plant strains.

Materials and Methods

The independent variable in this experiment was the different concentrations of sodium chloride. The dependent variable was the growth of the Arabidopsis roots and the protein production in the Arabidopsis roots. The negative control was 0mM of NaCl, and there was no positive control. Some of the constants were the temperature, the environment, the equipment used, and the

supplies used. There were five different concentrations. For each concentration there were five plates with five plants on each plate. This gave a total of 25 replications for each concentration. This experiment began by making a total of 600mL of MS agar using MS salts, sucrose, and distilled water. Growing plants on MS agar is a common technique taught in the advanced experimental design class that involves growing the plants on a medium that provides them nutrients and allows me to view their roots for easy analysis. A strip of pH paper was used to make sure the solution was around a pH of six. After heating on a hot plate, the 200mL of MS agar was then poured into a 250mL media bottle. The procedure was then repeated two more times to make a total of 600mL of MS Agar. About 200mL of MS Agar was then made for the following concentrations of sodium chloride: 0mM (negative control), 100mM, 125mM, 150mM, and 200mM. This was done using a similar procedure to making MS agar except adding extra amounts of sodium chloride to the agar solution. These extra values of sodium chloride that were added were determined using an equation. Once all of the agar was made, it was placed into an autoclave and sterilized.About 300 *Arabidopsis* seeds were then sterilized for culture using autoclaved distilled water and 30% bleach. They were placed in the freezer when they were done. After the agar was autoclaved, it was melted on a hotplate and poured into petri plates using sterile technique. Sixteen MS Agar plates were taken out and each plate had 10-12 *Arabidopsis* seeds placed on it to grow. The seeds placed on the plates had been sterilized and were placed on the plate one at a time using a micropipet. These plates were taped on the sides and stored vertically in a bin under a light cart to germinate for four days so that the roots would grow directly downwards. The plants were given time to germinate on regular MS agar plates before they were transferred so that they could germinate and grow uninterrupted for the first few days. After the germination period, the plants were transferred to new plates using tweezers so that they could now be exposed to the various added concentrations of sodium chloride in the MS Agar. There were five plates for each of the five concentration. With five plants per plate, this totaled about 125 *Arabidopsis* seeds that were transferred. The new plates were then rotated 90 degrees, taped on the sides, and placed back into the bins to be stored vertically under a light cart to grow so that the new root growth would grow at a new angle, making it easy to measure. For four days after the plants were transferred, each of the plant's change in root length was measured in millimeters using a ruler[3]. After taking observations, plant root samples from each concentration were crushed in Laemmli protein extraction buffer to create a sample to be run in a SDS-PAGE[4]. Multiple samples from each concentration were taken from different times because of lessons learned regarding the SDS-PAGE gels. These lessons will be explained later in the paper. When the SDS-PAGE samples were not being used, they were stored in a -80°C freezer. The samples were run on SDS-PAGE gels in PAGE buffer at 150V for 45 min- 1 hr. and 30min. Once the gels were run, they were then stained by sitting in Biosafe Coomassie stain for ten minutes and then destained using distilled water. Pictures were then taken to show how the banding patterns appeared[3]. It is important to note that a molecular weight marker was run on each gel to use as a point of comparison for the samples. Since there was extra time, a percent germination portion of the experiment was run. This was done by placing ten sterilized Arabidopsis seeds on each of ten MS agar plates containing various concentrations of NaCl, two plates for each concentration. The percent of germinated seeds was then recorded after four days. After all of the experiment was complete, all equipment and stations that were used were thoroughly cleaned and disinfected using sterile procedure. The data was analyzed in four ways. The mean root lengths for every day observations were taken for each concentration and the negative control was found. The standard deviation for each of the means for each concentration and the negative control were found. T-tests (2 type, 2 tailed) were performed between the control and each of the different concentrations for the final observations. The results from the SDS-PAGE gels were analyzed using a proteomic analysis. The proteomic analysis was completed by looking for the formation or deletion of banding patterns (a difference in the banding patterns) as compared to those of the negative control. These final observations were then analyzed by comparing them with each other to determine if the hypothesis should be accepted or rejected.

Results

The change in root length values for the different concentrations of sodium chloride in this experiment show very interesting trends. Figures 1 shows the change in root length (measured in mm) over the course of four days for the different concentrations of sodium chloride: 0mM (negative control), 100mM, 125mM, 150mM, and 200mM. There are standard deviation bars on all of the bars in the graphs to show the variability for each of the values that were graphed. In this graph, you can see that the change in root length at 0mM NaCl steadily and consistently increased throughout the course of the four day period. Since this was the negative control, these results were expected and the changes in root lengths were very large numbers, since the plants continued to grow in the absence of sodium chloride. The standard deviation bars got larger as the days went on, which makes sense as there was more variation in the change in root lengths the more time the plants had to grow. At concentrations of 100mM NaCl, 125mM NaCl, and 150mM NaCl, all showed similar trends in that the change in root length steadily and consistently increased throughout the course of the four day period but on a very different rate.

One difference to note is that at 125mM and 150mM, the standard deviation bars remained mostly constant for the four days and didn't increase. This just means that the error in the values for those concentrations was about the same all four days. At a concentration 200mM NaCl, you can see that the change in root length increased by very small increments and overall did not grow a great deal throughout the four day period. Overall, it can be observed that with each increased concentration of sodium chloride, the change in plant root lengths got shorter and the increments in which they increased also got smaller.

The percent germination portion of this experiment was very helpful in showing that once again, higher concentrations of NaCl inhibit plant growth. In Figure 2 you can see the percent germination of *Arabidopsis* seeds on different

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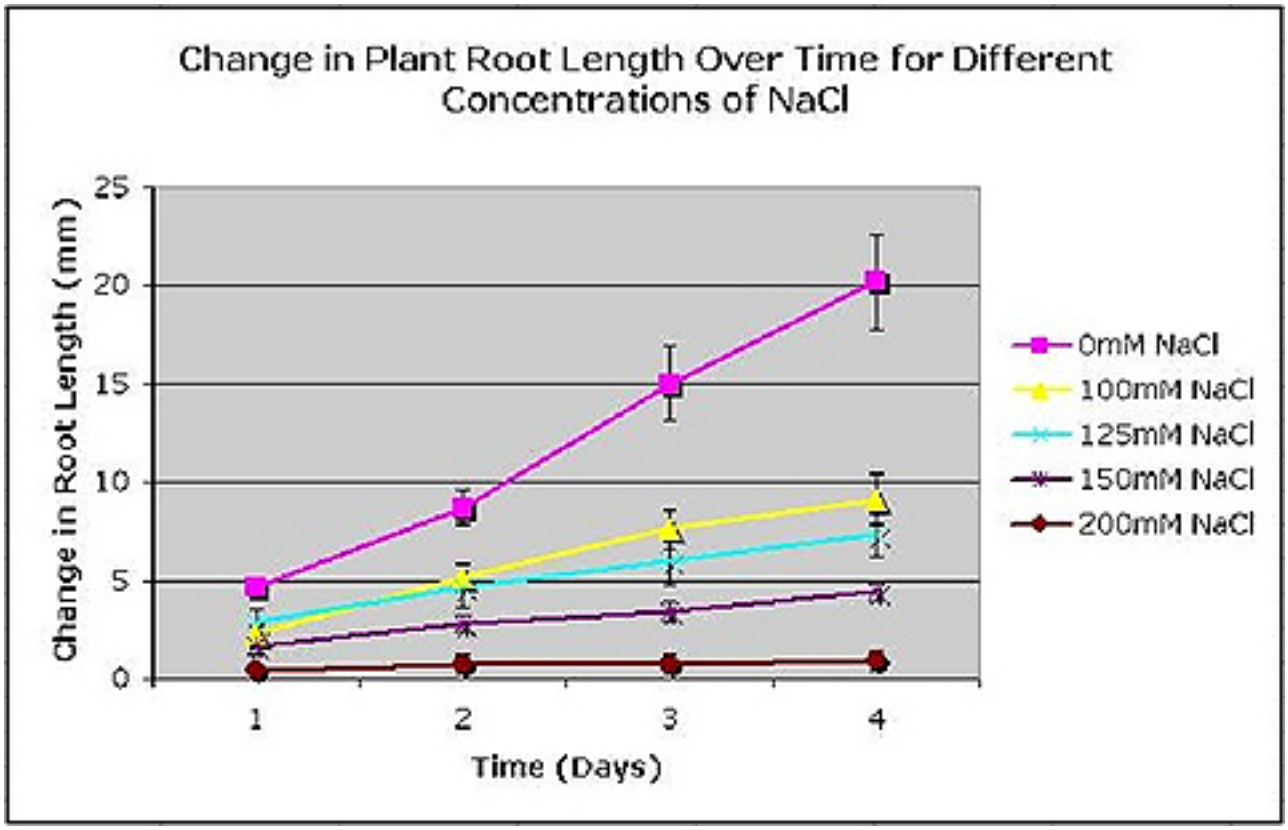

Figure 1. This graph shows the comparison between the changes in plant root lengths over a four day period for the different sodium chloride concentrations.

concentrations of NaCl after four days. The graph shows that the highest percent germination was for the negative control with 0mM concentration of NaCl and the lowest percent germination was for the 200mM concentration of NaCl. There was once again a steady decrease in the percent germination as the concentration of NaCl increased. The standard deviation remains consistent for the first four concentrations. The 200mM concentration of NaCl, however, happens to have a large standard deviation. The statistical analysis portion of this experiment had very conclusive results(Table 1). A t-test was performed between the control and each of the concentrations for the final day that measurements were recorded (Day 4). I decided to do the tests at this point because I thought it would be interesting to see how the final results compared to the control and how statistically different they were. I also decided to do a t-test between the control and each of the concentrations to see if each of the concentrations really had a statistically significant effect on the plant. To complete the t-test, I compared the 25 values of changes in root length for the negative control

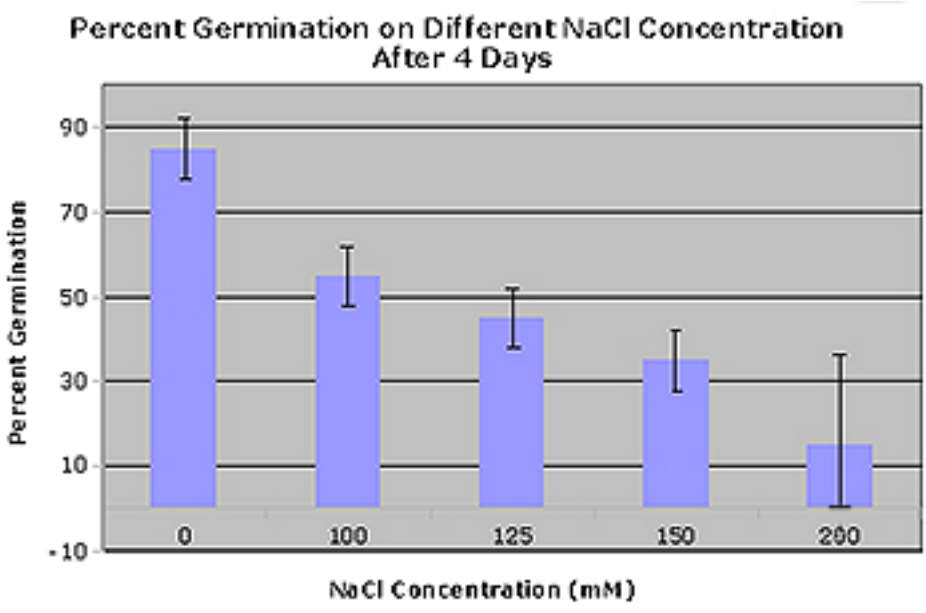

Figure 2. The average percent germination of *Arabidopsis thaliana* at different concentrations of NaCl after four days. length for each of the NaCl concentrations.

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on day 4 to 25 values of changes in root length for each of the NaCl concentrations. For each of the t-tests that I conducted, I found that the p- value was extremely small, less than 0.0001. Since the p- value is less than 0.05, this means that each of my concentrations compared to my control is significantly different. There is a less than 5% chance that my data sets are the same and the differences between them are most likely not due to random variation. In other words, the results that I got for each of the different NaCl concentrations is significantly different from the control values, showing that the NaCl concentrations had a significant effect on the plants and were not just minor effects. It shows that the changes between the data sets are significant.

The proteomic analysis completed for the different samples showed very interesting results. Figure 3 shows the SDS-PAGE gel using 0mM NaCl (Lanes 1-3), 100mM NaCl (Lanes 4-6). Figure 4 shows the SDS-PAGE gel using 125mM NaCl (Lanes 1-3), 150mM NaCl (Lanes 4-6). These results clearly show a band present in the three control plants that is not present in the other plants samples with NaCl concentrations. This shows that NaCl does in fact inhibit some sort of protein expression in plants. It cannot be determined specifically what kind of proteins are being inhibited, nor can a general size of the inhibited protein be given because the protein size marker is not spread out enough to be measured. The main important thing is that there is a band present in the control plants that is not present in any of the other plants. Something else to mention is that there were no samples from the 200mM concentration in the running of the last gels because the plants had died and were disposed of. It is predicted, however, that the results would have shown up the same with the 200mM concentration plants not having the extra band that was present in the control plant samples.

Discussion

Based on my results, I accept my hypothesis for a number of different reasons. My hypothesis stated that the plants would exhibit a number of different stress responses including shorter root length and an increased production in specific stress-responsive proteins when exposed to different concentrations of sodium chloride. My results did show a shorter root length as the concentrations of NaCl increased. So it would make sense that as a stress response to the NaCl, the plants exhibit a shorter root length. It could also be possible that the sodium chloride actually inhibits the proteins needed for the plant's roots to grow. This inference could be backed up by the results of the proteomic analysis that showed a protein band in the control plants that was not present in any of the NaCl concentration plants. So maybe instead of the production of specific stress-responsive proteins to help conserve water in the plant, instead is the inhibition of specific growth proteins having to do with energy production. This would make sense because if the plants cannot produce energy, then they cannot grow, thus inhibiting their root lengths. However, even though this proteomic analysis does not show the production of a new protein in the NaCl concentration plants, it is important to note that there was a proteomic response to the NaCl in the plants, whether it be an increase or decrease in the levels of a certain protein. In the future, I would like to run more SDS-PAGE gels so that I can get even clearer results with

Table 1. P-Values of t- Tests (2 type, 2 tailed)

Day 4 Negative Control vs. Day 4 100mM NaCl; p- value = 4.1 x 10-11
Day 4 Negative Control vs. Day 4 125mM NaCl; p- value = 1.2 x 10-13
Day 4 Negative Control vs. Day 4 150mM NaCl; p- value = 2.3 x 10-17
Day 4 Negative Control vs. Day 4 200mM NaCl; p- value = 3.5 x 10-21

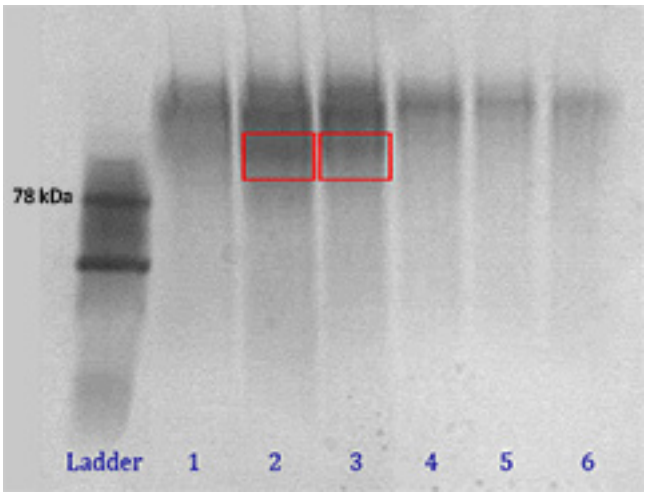

Figure 3. Banding patterns produced from the 0mM NaCl and 100mM NaCl samples run on a SDS-PAGE gel. Samples from left to right: Ladder, 0mM NaCl (Lanes 1-3), 100mM NaCl (Lanes 4-6). Unique band shown in red squares.

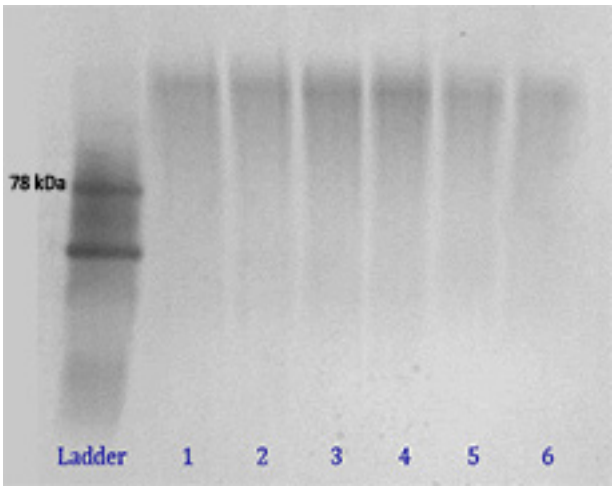

Figure 4. Banding patterns produced from the 125mM NaCl and 150mM NaCl samples run on a SDS-PAGE gel. Samples from left to right: Ladder, 125mM NaCl (Lanes 1-3), 150mM NaCl (Lanes 4-6).

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more banding patterns. This could potentially yield seeing a new protein expression in NaCl treated plants. Ultimately, I would accept my hypothesis since my results show that sodium chloride causes a shorter root lengths in plants and also a response on the proteomic level with the decrease in levels of a certain protein.

It is important to account for high levels of variation in the data. Some means had a standard deviation of 3, which is a relatively high number compared to the means. It means that there was a lot of variation in the data. Although in some cases there were high standard deviations, they were most likely caused due to outliers since for the most part, standard deviations were around the number one. To decrease the amount of variation in my data, I could have more replicates in a future experiment.

The results I found differed in light of the findings from my initial background articles. The results of the initial articles found that there with the stress of different sodium chloride concentrations being introduced to the plant there was the production of a specific protein to help manage the stress[1]. However, my results found that with the stress of different sodium chloride concentrations there was inhibition of a specific protein. In other words, I did not find that a new stress-responsive protein was produced. Instead, I found that the production of a specific protein was inhibited.

There are many possible sources of error in this experiment. The SDS-PAGE gels could have not run for long enough or run for too long. This would have caused the banding patterns on the SDS-PAGE gels to look different. For future experiments, I could do more background research on how to properly run a SDS-PAGE. The roots for the *Arabidopsis* plants intertwined at some points after a few days of growth, and so measurements on the change in root length may have been not accurately measured for some days. This would have affected my results in that I would have had different values. To solve this problem in the future, I could come up with a better method of making sure that the roots don't tangle such as putting separators into the agar.

There are a number of different ways that I could go on to continue this experiment in the future. I could test even more concentrations of NaCl or focus on a narrower range of concentrations. I could also try testing how different NaCl concentrations affect plants that are grown in soil versus agar, and see if it has a difference. Testing different NaCl concentrations on different types of plants could also provide interesting results. I could add more replicates to reduce the amount of error in my experiment. I could also try testing proteins in different areas of the plant, not just the roots and see if there are other protein chances in response to a NaCl stress response. All in all, there are different things that I could so to further continue this experiment.

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