Determining the Effects of Salt Stress on Switchgrass (*Panicum virgatum*) Growth in Tissue Culture Media

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INTRODUCTION

Switchgrass (*Panicum virgatum*) is a type of ornamental grass native to North America. It can grow three to six feet tall with a two to three foot spread. Switchgrass is tolerant and resilient and is the top leading biomass crop in the United States. Biofuels are an alternative fuel method that comes from living matter. There is debate over whether switchgrass should be used as a biofuel as it could potentially result in taking up land space that would otherwise be used for food crop. The basis of this experiment is to create a strain of switchgrass with the ability to grow in fields that are unusable for food crops.

MATERIALS AND METHODS

- Solutions were made using 42.4 g/L of Murashige and Skoog mixture with sucrose and agarose, 1µL/mL of vitamins, and the appropriate salt concentration. The salt concentrations 0, 0.25, 0.5, 0.75, 1.0, 1.15, 1.25, and 1.5 g NaCl/250 mL were tested. Each solution had the pH adjusted to 5.7. The solutions were then heated in a microwave until transparent.
- Tissue culture jars were used to transfer the solutions into. 50mL of the solution was pipetted into each jar.
- The tissue culture jars with the solution were then autoclaved and left to solidify under a sterile fume hood.
- Two days after preparing and autoclaving the media, lowland *Panicum virgatum* were planted in the media. 8-10 seeds were planted in each jar under aseptic conditions.
- The jars with seeds were then transferred to an incubator set at 25° C with a fluorescent light and 2 beakers filled with water to manage humidity.
- After 2 weeks had past, seeds that had germinated were recorded. Media that became contaminated was not included in the percentage of seeds germinated.
- Seeds that germinated were transferred into a new media after the 2-week period that was prepared with the same concentrations of the original media they were in.
- After 2 more weeks, the previously transferred plants were frozen.
- The RNA was extracted from frozen samples and converted to cDNA
- PCR with primers SAMDC, alpha-1, isomerase, and HSP22 was run on the cDNA samples in an attempt to isolate regulatory genes SAMDC and GAPDH, the gene for Alamo protein, AM-314/MS-155, and the rice salt stress gene at LOC4326471

FURTHER RESEARCH

After extensive genetic analysis of multiple concentrations, chemicals can begin to be added to media to help improve how the plant handles salt stress. Eventually this will lead to producing a salt tolerant strain of switchgrass. Future steps include running samples through qPCR and DNA sequencing. This will aid in choosing chemicals believed to have the strongest effect on targeted genes.

Upper Row:

Left: 0.0 g NaCl/250 mL
Middle: 0.25 g NaCl/250 mL
Right: 0.5 g NaCl/250 mL

Lower Row:

Left: 0.75 g NaCl/250 mL
Middle: 1.0 g NaCl/250 mL
Right: 1.5 g NaCl/250 mL

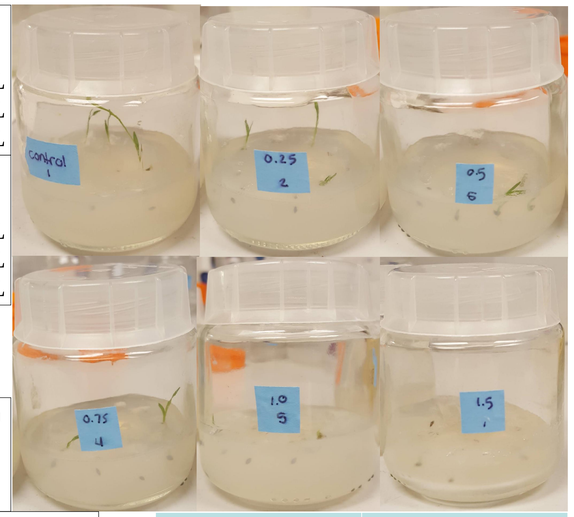
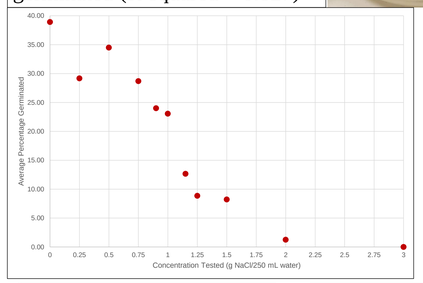
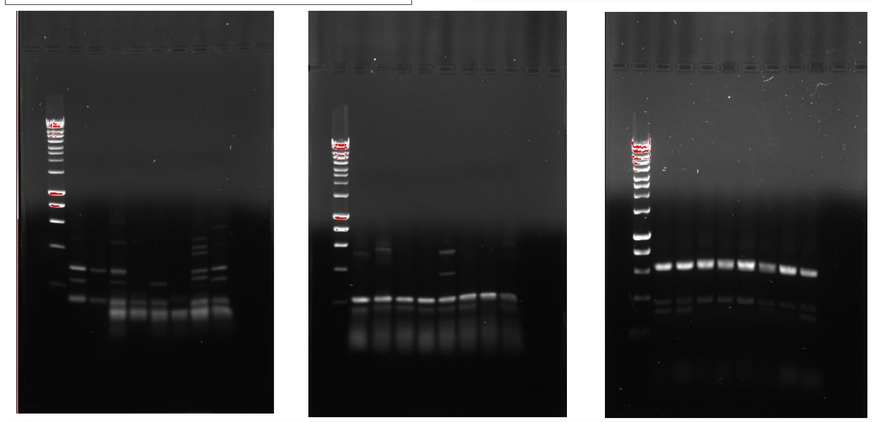


Figure 1: Determining correlation between concentration of NaCl and the percentage of seeds germinated (R-squared = 0.827)



Concentration tested (g NaCl/250 mL water)	Average Germination Rate per Jar
0	33.7%
0.25	29.3%
0.5	26.3%
0.75	27.0%
1.0	27.0%
1.5	6.7%



Left: Gel electrophoresis of 0, 0.25, 0.5, 0.9, 1.0, 1.15, 1.25, and 1.5 g NaCl/250 mL samples with SAMDC primer
Middle: Gel electrophoresis of 0, 0.25, 0.5, 0.9, 1.0, 1.15, 1.25, and 1.5 g NaCl/250 mL samples with HSP22 primer
Right: Gel electrophoresis of 0, 0.25, 0.5, 0.9, 1.0, 1.15, 1.25, and 1.5 g NaCl/250 mL samples with isomerase primer

REFERENCES

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DISCUSSION

Leaf Color and Growth:

- Yellowing occurred in the leaves of plants in the 0.5g NaCl/250mL concentration. This could have been due to salt stress, as the manner of how the leaves yellowed was very similar to how other plants yellow when under salt stress.
- The plants in the 0.75g NaCl/250mL media seemed to have an equal amount of leave yellowing as the plants in the 0.5g NaCl/250mL media. This shows plants in the 0.75g NaCl/250mL were also under stress from the salt.
- Plants grown in the 1.0g NaCl/250mL concentration had the most leaves that were yellow, suggesting further that it was under the most stress.
- As the concentration of salt increased, the plants would be seen to grow at a slower rate. This resulted in the final observed plants to be shorter and with less leaves for higher concentrations.

Germination Rate:

- The germination rate per tissue culture jar generally showed a downward trend. Until more trials are run, it cannot be stated that there is a significant difference between the concentrations seen in the table other than between 1.5 g NaCl/250 mL and the others.
- As expected, the graph shows a downward trend as the concentration of NaCl increases. There is a correlation of 82.7%. The steepest drop offs in germination rates occurs between 1.0 and 1.5 g NaCl/250 mL and 1.5 and 2.0 g NaCl/250 ml. These are concentration ranges that will be explored in future research.
- Error occurs due to contamination of the tissue culture media in the incubator from bacterial and fungal spores. Error also occurs due to dehydration causing the media to occasionally evaporate.
- Further research is being conducted in higher concentrations of sodium chloride to test the limits of switchgrass germination rates.

Salt Stress Expression in Genes:

- The primer SAMDC caused there to be differently sized gene fragments for samples that were the same except in salt concentration. This shows that SAMDC may not be a good choice to use when proceeding to qPCR.
- Primers HSP22 and Isomerase give clear and distinct bands, however, there does not seem to be much differentiation in expression under different salt concentrations. Different expression levels can be seen in the Isomerase primer gel, but it is not consistent with the increasing of the concentrations.