Using Sugar as a Germination and Growth Promoter of *Salvica Hispanica* Seeds.

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Abstract:

Seeds tend to undergo dormancy after being released from the parents. Seed dormancy is a condition that prevents germination in a specific period of time. When the environmental conditions are favorable, germination starts and the seed's embryo continues its growth (Swain et al., 1995). Germination begins when the seed absorbs water in a process called imbibition, then special enzymes that are responsible for seed growth are activated by water. This process is completed when the radicle (a part of the embryo that develops into the primary root) has grown out of the seed coat. Seed germination is controlled by many factors such as plant hormones, temperature, light, pH, etc. In this experiment, we tested the effect of sugar (sucrose) on the germination rate of chia seeds. We did so by having each group member place five chia seeds in four different containers (for a total of twenty seeds per student) containing a sucrose solution of 0%, 2%, 4%, or 6%. The chia seeds were then observed for fifteen days and data on sprouting and germination was collected every five days. After everyone had collected their data, we ran a one-way ANOVA test and a Tukey's test to determine whether our results were significant. Our results found that the growth rate means were significantly different. Specifically between the control and 4% sucrose solutions, and control and 6% sucrose solution. In addition, the germination rate showed a decreasing trend by increase in the concentration of sugar. .

Introduction:

Water is one the most prominent factors that determines how a seed germinates. This is because water is responsible for reactivating the seed's metabolism and is involved in all stages

of the germination process (Marcos-Filho, 2015). Sugars are metabolic substrates that play an important role in regulating a wide range of processes in plants during different phases of their development from germination to growth and finally senescence (Siddiqui et al., 2020). The sucrose concentration seeds are exposed to, can affect both their growth rate and germination rate. This is due to osmotic and ionic effects promoting changes in metabolic activities of the seed's cells, thus affecting plant growth. If the sucrose concentration is too high, the seed would be under stress and as a result the seed's growth rate would slow down or, in extreme cases, would end up in the seed dying (Stefanello et al., 2020).

Germination begins when the seed absorbs water in a process called imbibition, then special enzymes that are responsible for seed growth are activated by water. This process is completed when the radicle (a part of the embryo that develops into the primary root) has grown out of the seed coat. Increasing content of glucose has been shown to decrease the germination rate and height of sprouts in *Vigna radiata* which are possibly as a result of sugar increasing the inhibitory effects of ABA (Abscisic acid arrests embryo development by inhibiting water uptake and therefore seed germination) (Dewi, 2015). Once the embryo germinates from its seed, it begins to produce new organs such as stems and roots through the process of organogenesis. New roots grow from root meristems located at the tip of the root, and new stems and leaves grow from shoot meristems located at the tip of the shoot. The maturation of shoot is divisible into three phases: juvenile, adult non-reproductive, and reproductive. Glucose has also been shown to repress the gene responsible for controlling the transition from the juvenile phase to the adult phase in shoot development (Siddiqui et al., 2020).

This experiment was aimed to evaluate the effect of different concentrations of sugar water on the germination rate and growth rate of chia seed (*Salvia hispanica)*. Chia seeds were

chosen as the seed of choice because they don't require soil, they were accessible to all members of the group, and compared to other seeds (e.g. lentils and red beans) they have a faster sprouting rate. The sugar water was made using table sugar (sucrose), a disaccharide which is composed of two monosaccharides: glucose and fructose, in 4 different levels of concentrations: 0%, 2%, 4%, and 6%. The 0% solution serves as a control to compare to the effects of sugar water. The germination rate of the water-sugar applied and control seeds were measured after 5 days and the length of the germinated sprouts was recorded after 3 periods of 5 days, 10 days, and 15 days to analyze the effect of added sucrose solutions on the growth of the chia seed sprouts. Our null hypothesis is that the 4 different treatment groups with the different concentrations of sugar solution will show no difference in germination rate or the growth rate. The alternative hypothesis for our experiment is the higher the sucrose concentration (% sucrose in solution), then the germination rate (number of the seeds germinated and formed root) and the growth rate (length of the shoot (stem) of the sprouted seeds) would be lower because exogenous sucrose causes delays in germination and development of shoot based on the previously mentioned studies.

Methods:

Before starting the experiment, we each got 20 chia seeds and put aside a few teaspoons of granulated sugar (sucrose). We each made 4 different 100mL sugar concentration solutions (control (0% sugar), 2% sucrose solution, 4% sucrose solution, and 6% sucrose solution), using tap water, and labeled 4 cups with the 4 different concentrations of sucrose solution and poured the corresponding sucrose solution in each cup. In order to prevent cross contamination and the sucrose concentrations changing, we used 4 different spoons to mix the solutions. We then created the containers by cutting the bottom of another 4 plastic cups and

made sure to leave 2 cm. After doing so, we labeled the 4 containers (0%, 2%, 4%, and 6%). We then used the scissors to cut 8 equal pieces of the paper towel so they fit in the containers. Two pieces of paper towel were placed in each cup and that container's corresponding sucrose solution was poured onto the paper towels until both pieces of the paper towel were damp, around 1 tablespoon. In an effort to prevent contamination of the chia seeds, we washed our hands and made sure they were dry before placing 5 chia seeds on each paper towel. (The seeds were evenly distributed across the container). In an attempt to ensure that moisture stayed in the container, we wrapped each container with plastic wrap (also known as saran wrap) and then placed each container in a separate ziplock and labeled the ziplock with its corresponding sucrose solution concentration. We kept all the containers together in a tray by a window at room temperature so all the seeds would be exposed to the same environmental conditions. We visited the seeds every 5 days for 15 days and recorded our observations, how many seeds had germinated, and the length of the sprouts (mm).

After 15 days, we compiled our data and calculated the germination rate by finding the proportion of seeds that have formed roots. For analyzing the effect of sugar on growth, we calculated the difference between the seed length on day 5 and 15 for each seed and found the mean of this number in each sucrose solution group. In order to determine if our results were significant or not and if there is a statistically significant difference between the means, we did a one-way ANOVA test using the prism graph pad app. A Tukey's test was also performed as a part of our post-hoc analysis in order to find the significant between-group difference.

Results:

Under our experimental condition, the rate of seeds germinated (formed root) after 5 days was decreased from 80% for the 0% sucrose solution to 70% for the 2%, and 50% when 4% and 6% solutions were used. In order to measure the growth rate, a value corresponding to the

difference between the length of stems formed at day 5 and day 15 was calculated for each seed. Figure 1 shows significant differences between the average of this value measured for the seeds of each group (0%, 2%, 4%, and 6% sucrose solution). Based on the one-way ANOVA test result conducted between the length difference of day 5 and 15 for the 4 groups, assuming that our data is normally distributed, the distributions have equal variances, and our data are independent, the p-value is 0.0005 which shows a statistically significant difference among the means of the 4 groups since our p-value is less than 0.05 (significance level or alpha value). It indicates strong evidence against the null hypothesis, as there is less than a 5% probability the null is correct (and the results are random). Therefore, we reject the null hypothesis that treating the seeds with sugar-water does not have any effect on their growth and accept the alternative hypothesis that the 4 different treatment groups having different concentrations of sucrose in the solutions result in significantly different means of the growth rate of the chia seeds. We conducted a Tukey's test to compare the means of the 4 groups pairwise assuming that the observations that we are testing are independent within and among the groups, each group is normally distributed, and the within-group variance across the groups associated with each of the 3 means is equal. Tukey's test shows a significant difference when comparing the mean of the water treated (0%) seeds vs. the means of 4% solution treated seeds (with the mean difference of 25 (mm)), and the average of water treated seeds vs. the 6% solution treated seeds (with the mean difference of 28.83 (mm)) demonstrating that the 0% solution (water) resulted in the highest growth rate for chia seeds.

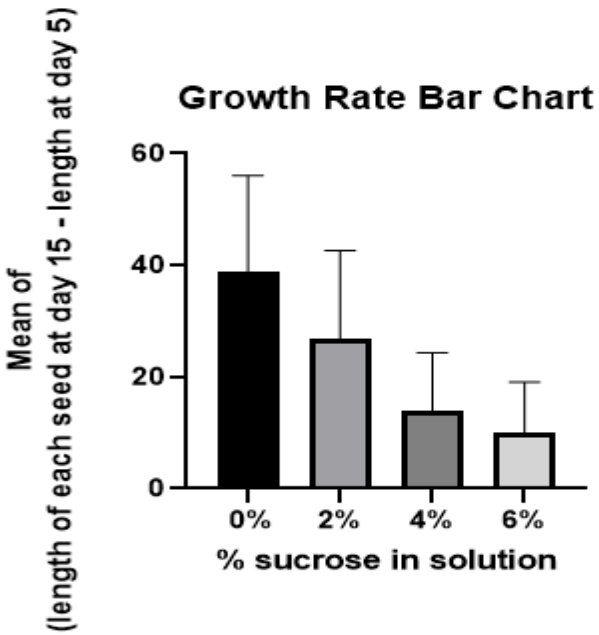

Fig. 1. The bar chart shows the length grown between day 5 and day 15 for each group of seeds. A decreasing trend can be seen in the growth rate by increase in sucrose solution with the 0% solution having the fastest rate (average difference 38.83 (mm)). Error bars show standard deviation.

Discussion:

We observed a trend of decreasing germination rate as sucrose concentration increased. From our results, we can reject our null hypothesis and conclude that there was a significant difference in the mean growth rates of the different treatment groups. An additional Tukey's test concluded that the control and 4% groups were significantly different similarly the control and 6% groups were significantly different. In general, we observed a trend of decreasing sprout length with increasing sucrose concentration.

Our observation of decreasing germination rate with increasing sucrose concentration used is in line with previous studies. For instance, Price et al. and Dewi both find that sugars are used as signalling molecules during seedling development (2003; 2012). Specifically, sugar plays a role in inhibiting seedling development by increasing the effect of the growth inhibitor abscisic

acid (ABA). These findings possibly explain the germination results of our experiment. Moreover, we observed that the 4% and the 6% treatment groups had the same germination rate. This may be due to the small sample size used for each treatment group as only 5 seeds were used for each treatment. Furthermore, Rai et al. reported a germination rate of 68% at 3% sucrose solution, while a 5% sucrose solution yielded a 30% germination rate in guava seeds. This shows that germination rates may vary between a threshold, and that the 4% and 6% treatment group germination rates may not be entirely represented by the small sample size used.

Two possible explanations for our results are triggering of senescence and osmotic stress. First, the observed trend and significant difference in growth means between treatment groups could be due to the onset of plant senescence by exposure to sugars. A study by Pourtau et al. found that the senescence gene, SAG-12, was triggered in *Arabidopsis* (common name: thale cress) when exposed to a light dosage of sugar (2006). Dosages in our experiment were relatively low and similar to Pourtau et al.'s experiment, possibly explaining how growth was halted at higher concentrations (2006). As a result, there could have been an onset of senescence in the chia seeds, triggered by light dosages of sucrose. Second, osmotic stress can also suppress plant growth. Osmotic stress has been seen to affect protein synthesis in plants and cause protein denaturation (Levitt, 1972; Burke et al., 1988). Since we monitored growth for up to 15 days, possible evaporation could have occurred to cause osmotic stress on the sprouts. In addition, an increase in sucrose concentration means there is a decrease in water concentration in the solution. This means that higher sucrose concentration could have induced osmotic stress in the sprouts, resulting in a trend of decreased growth with increasing sucrose concentration.

Although our results were statistically significant, there are limitations to the experiment. No specific brand of chia seed was agreed upon, and both white and black chia seeds were used. In one study, black chia seeds were found to have higher protein, ash, and moisture content in the seed, which means that this could result in different growth rates or germination rates or seeds (Tuncil & Celik, 2019). Moreover, the seeds were grown in three different environments, meaning temperature, light availability, and humidity may vary between the three environments. These abiotic factors are important to seed growth, for instance, the optimal temperature for chia seed growth is 25°C (Nadtochii et al., 2019).

In addition to limitations, the experiment should be repeated to eliminate sources of uncertainty. Some possible sources were a lack of standardization of equipment used. Measurement of sugar concentrations were done using volume measurements (measuring spoons) or a scale. This may have resulted in a discrepancy between the trials. Furthermore, those using a kitchen scale had difficulty measuring a small quantity of sugar. It has also been shown that sugar also affects germination rate due to sugar increasing inhibitory effects of ABA (Dewi, 2015). Due to this effect, some seeds sprouted later than others, meaning that some sprouts grew for a longer period of time than other shoots. This delay was not accounted for.

To eliminate the sources of uncertainty, standardizing the type of equipment used such as same colour chia, same brand, and same measuring tools can reduce the uncertainty due to the use of different equipment. Moreover, the trials should be conducted in the same environmental conditions, preferably in the same room, to eliminate uncertainty from exposure to different abiotic factors. Lastly, a new method should be developed to account for the delay of germination due to added sugars. Specifically, we should sprout seeds using plain water first, then measure the starting length of each sprout. Then, transfer each seedling into the appropriate treatment and continue to monitor the growth as previously outlined, and record

lengths as we monitor. At the end of the monitoring period we will take the difference between the start length and end length. This would more accurately give growth rate measurements and accounts for the delay due to germination.

For future experiments, more trials should be conducted in the same environment, using the same measurement protocols. By conducting more trials, we can more rigorously test the null and alternative hypotheses. Furthermore, future iterations of the experiment can be conducted using soil to see how different environments affect growth rate. Information obtained from this study can potentially be used to further investigate the health benefits of chia seeds and sprouts and possible applications of use (Mohd Ali et al., 2012). For instance, chia seeds have been found to have positive benefits on human health such as cardiovascular diseases, diabetes and metabolic syndrome (Guevara-Cruz et al., 2012). Moreover, this information can be used to determine optimal growing conditions for chia crops to increase production, as chia seeds have multiple applications in the food industry. For example, the crop can be used in animal feed, flour in baking products, and supplements (Ali et al., 2012). Overall, more variations of this experiment should be conducted to extract more information on the optimal growth conditions for chia seeds.

Conclusion:

The rate of growth of chia seeds due was observed to decrease with increasing sugar concentration solutions. From our statistical analyses, we conclude that the mean chia sprout growth rate differed significantly between treatment groups. Specifically, the control and the 4%, and control and the 6% growth rate means differed significantly. One possible explanation of this observation is that low sugar concentrations and sufficient light exposure have been seen to trigger senescence (Pourtau ete al., 2006). Another possible explanation could be the triggering of senescence through osmotic stress, since the concentration of water could have

been reduced through evaporation or increased sugar concentration (Levitt, 1972; Burke, 1988). Lastly, this information can be used to supplement further research of optimal growth of chia sprouts, as chia seeds provide a myriad of health benefits (Ali et al., 2012; Guevara-Cruz et al., 2012).

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Appendix:

Data table:

		Length After 5 days (mm)	Length After 10 days (mm)	Length After 15 days (mm)	The length difference between day 15 and 5 for each seed (mm)
Control	Seed 1	3	20	25	22
	Seed 2	5	25	28	23
	Seed 3	10	30	35	25
	Seed 4	10	30	40	30
	Seed 5	0	15	23	23
	Seed 6	5	11	57	52
	Seed 7	5	10	68	63
	Seed 8	4.5	10.5	61	56.5
	Seed 9	6	11	61	55
	Seed 10	0	0	0	
	Germination-rate (number of seeds germinated at day 5)	80%			
	Seed 1	3	15	18	15
	Seed 2	3	20	22	19
	Seed 3	5	20	22	17

	Seed 4	5	20	23	18
2%	Seed 5	10	20	30	20
	Seed 6	5	9	50	45
	Seed 7	3	11	57	54
	Seed 8	0	0	0	
	Seed 9	0	0	0	
	Seed 10	0	0	0	
	germination-rate	70%			
	Seed 1	3	10	17	14
	Seed 2	0	8	14	14
	Seed 3	0	0	3	3
	Seed 4	0	0	0	0
4%	Seed 5	0	0	0	0
	Seed 6	3	9	24	21
	Seed 7	5	9	30	25
	Seed 8	2	5	28	26
	Seed 9	4.5	9.5	26	21.5
	Seed 10	0	0	0	
	germination-rate	50%			
	Seed 1	3	8	16	13
	Seed 2	3	6	8	5
	Seed 3	0	3	3	3
	Seed 4	0	0	0	0
6%	Seed 5	0	0	0	0
	Seed 6	4	6	26	22
	Seed 7	3	7	20	17

	Seed 8	3	9	23	20
	Seed 9	0	0	0	
	Seed 10	0	0	0	
	germination-rate	50%			

Appendix A. Raw data

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ANOVA summary	
F	8.081
P-value	0.0005
Significant diff. among means (P < 0.05)?	Yes
R squared	0.4553

Appendix B. The one-way ANOVA test summary

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value	
0% vs. 2%	11.98	-6.646 to 30.60	No	ns	0.3163	A-B
0% vs. 4%	25	7.580 to 42.42	Yes	**	0.0027	A-C
0% vs. 6%	28.83	10.88 to 46.79	Yes	***	0.0008	A-D

2% vs. 4%	13.02	-5.599 to 31.65	No	ns	0.248	B-C
2% vs. 6%	16.86	-2.268 to 35.98	No	ns	0.0992	B-D
4% vs. 6%	3.833	-14.12 to 21.79	No	ns	0.9368	C-D

Appendix C. The Tukey's multiple comparison test