The little green onion that could: Determining the effect of varying salinities on

the rate of regrowth of A. fistulosum

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Abstract

Green onion (*Allium fistulosum*) regrowth is a method in which an individual can take one stalk and re-use it many times over, so it makes sense that one aspect of efficient green onion use would be maximizing the rate of regrowth. In this experiment, it was hypothesized that green onions regrown in high levels of NaCl will exhibit a lower rate of stalk and root regrowth than green onions regrown in low or no levels of NaCl. Two samples of *A. fistulosum* were assigned to one of three treatment groups (aqueous solutions of 0 mM NaCl, 10 mM NaCl, and 70 mM NaCl) for a total of six samples. Samples were allowed to grow for 18 days, and rates of regrowth were measured (mm) every 4 to 5 days using a tape measure. The obtained data underwent statistical analysis using a one-way ANOVA test, and the reported p-values were $p_{stalk} = 0.1493$ and $p_{root} = 0.1499$, both > 0.05. Therefore, the results of this study fail to indicate that there does exist a true difference between the means of the three treatment groups in relation to stalk regrowth and root regrowth, contrary to what evidence from the literature may suggest. This result could be explained by the small sample size (n = 2), or the selection of 10 mM NaCl as the low concentration treatment. A future experiment, with these experimental limitations fixed, is strongly recommended in order to verify the findings of this paper.

Introduction

Green onions (*Allium fistulosum*) are a widely used ingredient in the culinary arts, especially in East Asia (Rabinowitch, and Currah). Due to its high prevalence in cuisines, it would be in many individuals' best interests to maximize the efficiency of *A. fistulosum* usage. One method is to place the bulbous roots of green onions in water, which when left exposed to sunlight, leads to regrowth of the green onion stalk (Greaves). It may be the case that differences in the mineral content of water could lead to differences in the regrowth of *A. fistulosum*.

In a previous study, the common onion (*Allium cepa*), widely known as white or red onion, was placed in water treatments of various NaCl concentrations, ranging from 0 mM to 125 mM (Chang, and Randle). The results of the experiment indicated that samples in the treatment with a higher concentration of NaCl had lower average final mass than samples in the treatment with a lower

concentration of NaCl. However, there has been little previous research studying how NaCl concentration in water affects regrowth of *A. fistulosum*, and therefore this will be the focus of this paper.

In this study, it was hypothesized that if increased concentrations of NaCl in growth medium are detrimental to green onion regrowth, then a lower rate of regrowth in stalk and root will be observed in green onions regrown in high levels of NaCl compared to green onions regrown in low or no levels of NaCl. This line of logic is parallel to the results of the aforementioned study, conducted by Chang and Randle.

<u>Methods</u>

Three different concentrations of NaCl were chosen to be the treatment groups for this experiment: 0 mM, 10 mM, and 70 mM. The 0 mM treatment (control) was prepared using only unfiltered tap water. The 10 mM and 70 mM treatments were prepared using unfiltered tap water and table salt, as well as volumetric measuring equipment. These treatments were produced by a series of dilutions, starting from a initial solution of 1 M NaCl. No particles of NaCl were visible in the final solutions, indicating full dissolution of the appropriate amount of NaCl in water. The varying solutions were placed in 6 open glass jars with base area 12 cm² and height 9 cm.



Fig. 1. Serial dilution flowchart.

Two samples of green onion were assigned to each treatment group for a total of six green onions. The lengthy stalks of green onions were severed and disposed. The roots of each sample were trimmed so that the majority of roots were approximately 15 mm in length. Stalk length (mm), as well as root lengths (mm) of the three longest roots were recorded for each sample using a small tape measure prior to placement into the treatment jars. To ensure that the samples were not completely submerged, approximately 75 mL of solution was transferred into each jar. Treatment jars were placed a windowsill which received ample sunlight, and samples were allowed to grow for a period of 18 days. Measurements and observations were taken every 4 or 5 days; measurements were made with a tape measure and recorded (mm). Stalk length was measured, as well as the lengths of the three longest roots. Each sample was carefully taken out of the treatment jar, then placed on a paper towel bed for measurement. Each treatment solution was replaced by newly created solutions of corresponding concentration every 7 days.

Upon completion of data collection, the length of regrown stalk and roots were calculated for each sample. The length of regrown root was calculated as the mean of the length of regrown root for the three longest roots. A one-way ANOVA test was then applied using GraphPad Prism, a statistical analysis and graphing software program.

Results



NaCl concentration vs. stalk regrowth

Fig. 2. Mean stalk regrowth (mm) of each treatment group. p-value for stalk regrowth = p_{stalk} = 0.1493. n = 2 for each treatment. Error bars represent standard deviation.

NaCl concentration vs. root regrowth



Fig. 3. Mean root regrowth (mm) of each treatment group. p-value for root regrowth = p_{root} = 0.1499. n = 2 for each treatment. Error bars represent standard deviation.

From the one-way ANOVA test, it was concluded that there is no significant difference between the means of each group for both stalk regrowth and root regrowth. Moderate standard deviation was observed for the regrowth of most treatment groups. A relatively small standard deviation (4.24 mm compared to 34.65 mm and 30.41 mm) was calculated for the stalk regrowth of the control group, and a relatively large standard deviation (21.21 mm compared to 7.78 mm and 4.24 mm) was calculated for the root regrowth of the 10 mM group. The stalk regrowth means for each group were as follows: 211 mm for the control group, 229 mm for the 10 mM group, and 158 mm for the 70 mM group. The root regrowth means for each group were as follows: 55 mm for the control group, 48 mm for the 10 mM group, and 20 mm for the 70 mM group.

Discussion

The one-way ANOVA test resulted in p-values of 0.1493 for stalk regrowth and 0.1499 for root regrowth. Since both values are greater than the significance level of 0.05, it can be concluded that a statistically significant difference does not exist. Thus, we fail to reject the null hypothesis and consequently fail to support the alternate hypothesis. The results fail to support the existence of true differences between the means of each group, and the differences in our observed results are most likely due to chance. The data also fail to support the initial prediction that green onions regrown in water of high NaCl concentration will show the smallest rate of regrowth, which would be aligned with the findings of Chang and Randle in their study of A. cepa. In contrast, the theory that there is no relationship between the rate of green onion regrowth and NaCl concentration in growth medium is supported by the results. This is a peculiar result, given that there is much literature regarding the negative effects of NaCl on plant growth. If Na⁺ and Cl⁻ ions are present in high concentrations, plants may uptake these ions instead of essential plant nutrients such as phosphorus and potassium (Bayer, and Njue). Phosphorus is an essential nutrient for plant growth, as it is required for the synthesis of key molecules such as nucleic acids, ATP, and phospholipids; phosphorus is also involved in vital processes such as energy transfer, photosynthesis, and nutrient movement (Khan, and Zaidi). Potassium is also essential for plant growth because it acts as an activator for numerous important enzymes involved in processes such as photosynthesis, protein synthesis, and sugar transport (Xu et al.). On the other hand, an excessive accumulation of Cl⁻ ions can result in an interference of normal photosynthetic function, which would stunt growth (Bayer, and Njue). In a similar vein, plants placed in a medium of high osmolarity undergo osmotic stress and exhibit reduced cell expansion in root tips and as young leaves (Munns, and Tester). Given this framework, the observed lack of true differences between the three treatment groups is quite odd, as it is unexpected when compared to results of previous studies with similar objectives.

A plausible explanation of the lack of true differences between treatment groups could be due to sources of variation or error within this particular experiment. Most importantly, note the fairly large standard deviations present in Fig. 1 and Fig. 2: this holds for all but one reported mean value from above. This is most likely due to the limited sample size (n = 2) in each treatment group. With such a small sample size, the presence of outliers greatly skews the data and results in large values of standard deviation. Having a greater sample size would decrease the impact of outliers on the variation in stalk and root regrowth, thus reducing the standard deviation present: this may have led to statistically significant results as one would have expected. The presence of contaminants in the table salt used should not have affected the results. As mentioned above, osmolarity, which is a measurement of the number of solute particles present in a liter of solution (regardless of the nature of the solute), is inversely proportional to the rate of plant growth. Therefore, contaminant particles should have had no effect on the osmolarity and very little effect on the NaCl concentration of the treatment solutions. As such, the regrowth of A. fistulosum should not have been affected significantly by the presence of contaminant particles in the table salt, if there were any present. The inability to determine true differences between the means of treatment groups could also stem from the fact that perhaps a concentration of 10 mM NaCl is not high enough to result in a significant difference in root and stalk regrowth given such a small sample size when compared to the control (0 mM NaCl). In a future experiment, it could be recommended to use a larger number of samples per treatment group, or increase the 'low NaCl' treatment level to 25 mM NaCl, or even both of these measures, in order to reduce the amount of variability or uncertainty present in the experiment.

Conclusion

The results of the study were not statistically significant: that is, a true difference was not found to exist between the treatment groups in relation to mean stalk regrowth and mean root regrowth. This contradicted the given hypothesis that *A. fistulosum* regrown in a medium of high NaCl concentration would exhibit a lower rate of regrowth than *A. fistulosum* regrown in a medium of low or no NaCl concentration. Although the results of the study indicate that individuals who wish to regrow *A. fistulosum* need not worry about the NaCl concentration of the growth medium, a future experiment with larger sample size and different concentration level treatments is strongly recommended to verify these findings.

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<u>Appendix</u>

Res	115 for dut. 27, 2000				
#	Stalk length (mm)	Root (mm)	Root 2 (mm)	Root 3	S (mm)
T	60	16	16	15	
2	53	18	16	16	
2	55	6	17	16	
	51	16	15	15	
3	53	13	1.5	14	
h	57	15	17	16	
*				· · · · ·	
	Roots not cut to	10mm bec	muse it looke	dlike	It would be
	Roots not cut to too short.	0 10mm bec	muse it looke	d like	It would be
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Nov	Roots not cut to too short. 1.2020	0 10mm bec	use it look	e d	P-12(mm)
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Rosults Nav. 5	(con ² t.) ZOZO				
#	Stalle (mm)	Root (mm)	Root Z (mm)	Root .	3 (mm)
	182	57	22	Se	>
7	159	35	29	3	2
3	210	69	65	40	1
4	195	32	34	4	0
5	105	29	27	3	0
6	110	20	22		व
Nov.	9,2020				
#	Stalk (mm)	- Rost Cinn	a) $Rost 2$	(mm)	Root 3 (m)
7	+ 241	64	>9		58
6	237	45	42		41
5	265	60	69		74
4	236	45	43		40
>	163	33	36		32
6	159	31	22		31

Nav. 14, 2020							
#1	Stelk (m)	Root I (mm)	Root 2 (mm)	(Root 3 Cmm)			
I	274	74	76	77			
2	261	68	65	64			
3	308	BI	30	77			
4	255	50	SI	43			
S	232	35	34	36			
6	143	34	32	37			