Effect of Salinity on the Growth of Welsh Onions (*Allium fistulosum*)

Authors: Balkirat Mahal, Harman Sandhu and Sandeep Singh

Abstract
This study aimed to investigate how varying concentrations of salt will affect the growth of Welsh onions (*Allium fistulosum).* In this experiment, using Sodium Chloride (NaCl), four different treatments (0, 50, 100, 200 millimolar (mM)) levels were made. Three onions were placed in each treatment level in different cups and were monitored for a total of two weeks. A significant decrease in the stem length of the Welsh onions was seen as the NaCl concentrations increased, this is seen as after the two week period the 0 mM treatment level stem grew to an average length of 22.8 cm whereas the 50, 100 and 200 mM grew to an average length of 16.6, 14.1 and 10.9 cm respectively. Similar trends were seen in the growth of the roots as the average length for the 0 mM treatment level was 7.8 cm whereas the average length for the 50, 100 and 200 mM was 4.7, 3.6 and 1.5 cm respectively. From this, it can be understood that as the concentration of NaCl increases, the growth of the stems and roots will be reduced. Overall, from this experiment, we can say that NaCl is a compound that, at high concentrations, inhibits and hinders the growth capabilities of Welsh onions.

Introduction

With the world population expected to increase by 2 billion by the year 2050, soil salinity is becoming an immense obstacle for agriculture (Gupta & Huang, 2014). Currently, 20% of the agricultural land around the world is affected by salt stress and this percentage is expected to increase over the coming years (Gupta & Huang, 2014). Sodium Chloride (NaCl) is considered to have one of the most detrimental effects on plants as the accumulation of Na^+ and Cl^- ions inside the tissues of plants results in severe ion imbalance (Gupta & Huang, 2014). This prevents the uptake of essential minerals such as Potassium ions (K^+), leading to lower productivity, growth and sometimes even death (Gupta & Huang, 2014). Onions are classified as a salt-sensitive crop (Chang & Randle, 2004); hence a lot of research has been done on investigating the impacts of NaCl on onion growth. An experiment conducted by Chang and William looked at whether the growth of bulb onions (*Allium cepa*) changed as the concentration of NaCl varied (2004). They found that as the concentration of NaCl increased, a drastic decrease in the growth of the bulb onions was seen. Similarly, a study run by Singh and Roy looked at how well bulb onions can handle an increase in the concentration of NaCl (2015). Through this experiment, they found that up to 150 mM the bulb onions can do a decent job at resisting the NaCl-induced stress but at concentrations above that (200 mM) the plant is unable to do so as an extreme decrease in growth is seen. While these publications do a great job at portraying how NaCl affects the growth of onions, they focus solely on bulb onions (*Allium cepa*). In our experiment, we decided to look at how different concentrations of NaCl (0, 50, 100, 200 mM) affect the growth of Welsh onions (*Allium fistulosum*). Investigating this is important as many types of onions are grown across the world and understanding how NaCl affects each type is crucial. The goal of this experiment was to look at how different concentrations of salt impacts the growth of Welsh onions. The hypothesis of this experiment is that if the concentrations of NaCl increases then the growth (root and stem length) of the Welsh onions will decrease.

Methods

In this experiment, Welsh onions were used to test the impact of salt on their growth. Table Salt (NaCl) was used and four solutions of different NaCl concentrations were prepared. The concentrations used in this experiment were 0 mM (0g NaCl/L of water, no salt added), 50 mM(2.7g NaCl/L of water), 100 mM (5.8g NaCl/L of water) and 200 mM(11.7g NaCl/L water). These concentrations were chosen because previous studies, including the one by Singh and Roy in 2015, have done similar experiments using concentrations ranging from 0 to 200 mM. In our experiment, each onion was cut to 8.5cm and the roots were left uncut. The initial lengths were recorded for the stems and roots. Each onion was placed in a plastic cup with 1cup of water for each treatment. Furthermore, 3 replicates were done per treatment and the cups were placed in an area that received even sunlight.

Image 1. This image shows how the onions were cut and placed into each treatment level

We then monitored and recorded the growth of both the root and stem lengths daily. The method used to measure the stem and root was firstly taking the onion out of the aqueous solution and then via a ruler measuring the longest strand of the root (root measurement) and then measuring the rest of the onion (stem measurement). This was done for a total of 14 days from which a large data pool was made. A one-way ANOVA and a Tukey's multiple comparisons test was done to test for significance.

Results

The one-way ANOVA test for the length of the onions resulted in a p-value of 0.003 and a p-value of less than 0.001 for the root length data. The line graphs below show the growth data for each treatment over 2 weeks. Each treatment had 3 replicates of onions. The root and onion length were averaged out for each day. Figure 1 shows the data for the onion length and figure 2 shows the data for the root length for each concentration of salt. The mean lengths and standard deviations are provided in the figure legends below. Raw data and Graphpad sample analyses are shown in the Appendix. The onions subjected to low concentrations of salt grew at higher rates and were seen to uptake more water than those in high concentrations.

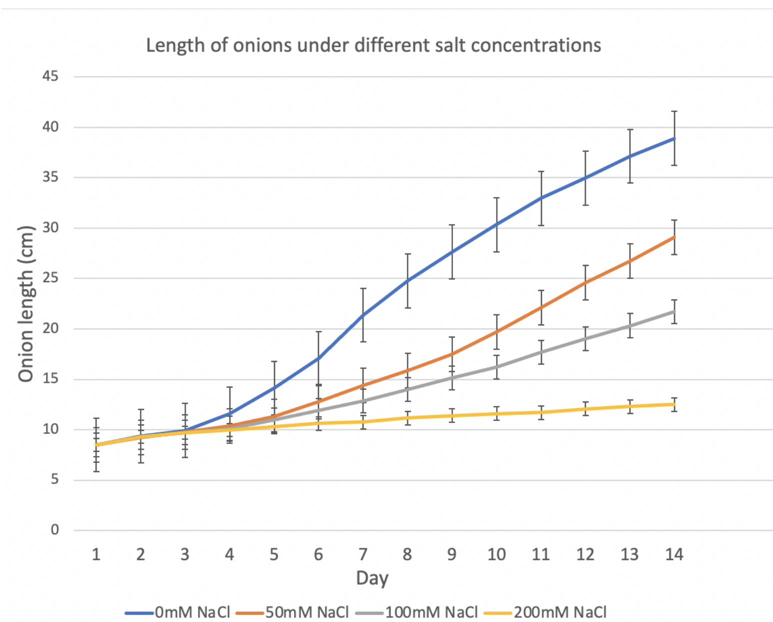

Figure 1: Average onion length for each treatment(0mM, 50mM, 100mM, and 200mM NaCl) is shown. Sample size is n=3 for each treatment. P-value =0.003.Error bars show standard error for each treatment.

Treatment 1(0mM): mean onion length=22.8cm, SD=11.0cm
Treatment 2(50mM): mean onion length=16.6cm, SD=6.9cm
Treatment 3(100mM): mean onion length=14.1cm, SD=4.3cm
Treatment 4 (200mM): mean onion length=10.9cm, SD=1.2cm.

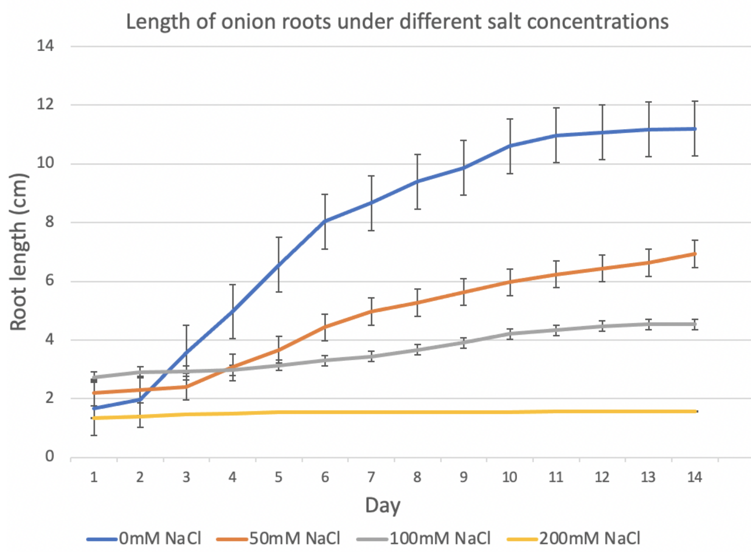

Figure 2 : Average root length for each treatment(0mM, 50mM, 100mM, and 200mM NaCl) is shown. Sample size is n=3 for each treatment. P-value <0.0001.Error bars show standard error for each treatment.

Treatment 1(0mM): mean root length=7.8cm, SD=3.5cm
Treatment 2(50mM): mean root length=4.7cm, SD=1.7cm
Treatment 3(100mM): mean root length=3.6cm, SD=0.7cm
Treatment 4 (200mM): mean root length=1.5cm, SD=0.1cm.

Discussion

With the results obtained from this study, it can be concluded that when the concentration of NaCl increases, the growth of Welsh onions (*Allium fistulosum*) is inhibited. The p-value (significance level) obtained from the statistical analysis of both root ($p < 0.0001$) and onion length ($p = 0.003$) have a p-value less than 0.05, therefore these results are significant. Through this statistical analysis, the alternate hypothesis is accepted and the null hypothesis is rejected. The overall effect of NaCl observed was that the increase in the concentration of NaCl resulted in a decrease in the growth of the onions. Also, as the concentration of the NaCl was increased, the average growth of both the root and the onion stem length would decrease evidently. A Tukey's multiple comparisons test was also conducted (see appendix) where the average difference between 0 mM and 100 mM are significant and the average difference between 0 mM and 200 mM were also significant. This analysis tells us that the concentration of NaCl of 100 mM and 200 mM compared with 0 mM (tap water) are statistically significant whereas the concentration of 50 mM compared to 0 mM (tap water) did not have a statistical difference. This test further supports the alternate hypothesis of increasing the concentration of NaCl will decrease the growth of both the root length and the onion length. The results obtained in the experiment correlate with some experiments done in the past. In a 2004 study done by Chang and Randle, it was found that as the concentration of NaCl was increased the growth of the onion would decrease. In this experiment, it was found that when the onion and the stem were in the 200 mM NaCl solution they did not grow a significant amount. Especially the roots, they grew for the first couple of days of the trial but after those days, there was no growth in the root length seen in the 200 mM solution of NaCl. Moreover, in another study done by Singh and Roy in 2015, similar results were seen. In their experiment, it was found that the onions (although different from the ones done in this experiment) had a drastic fall in growth after the concentration of NaCl was greater than 200 mM. In the experiment done here, the growth of onions in the 200 mM NaCl solution (mean onion length:10.9 cm, mean root length:1.5 cm) was drastically lower than the 0 mM NaCl solution (mean onion length:22.8 cm, mean root length:7.8 cm). Some sources of error in this experiment can be; the cups used in this experiment were contaminated, this can negatively affect the results of the experiment as these other substances present may influence the growth of the onions. Furthermore, the curvature of the onions growing may have affected the results, this is a possibility as when onions grow they do not always grow straight and this can pose a problem when measuring the growth of the onion. Therefore, in future studies, these sources of error can be addressed by washing and drying the cups thoroughly. The curvature problem can be fixed by using a string to measure the size of the onion and then measure the string using the ruler because the string can be bent along the onion.

Conclusion

In conclusion, the results obtained in this experiment help justify that increasing the concentration of NaCl in the water will decrease the growth of the onion and its stem (*Allium fistulosum*). Through these results, the p-value observed for both growth of the root length ($p < 0.0001$) and onion length ($p = 0.003$) have a p-value less than 0.05, therefore these results are significant. This allows us to accept that the alternate hypothesis is correct and reject the null hypothesis.

Acknowledgements

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References

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Singh, D., & Roy, B. K. (2015). Salt stress affects mitotic activity and modulates antioxidant systems in onion roots. *Brazilian Journal of Botany*, 39(1), 67–76. https://doi.org/10.1007/s40415-015-0216-0

Appendix

1. Onion (stem) length averaged data and one-way ANOVA and multiple comparison analysis from GraphPad

	Group A	Group B	Group C	Group D	Group E	Group F
	0mM NaCl (Average Onion length in cm)	Onion 50mM NaCl (Average Onion length in cm)	Onion 100mM NaCl (Average Onion length in cm)	Onion 200mM NaCl (Average Onion length in cm)	Title	Title
	Y	Y	Y	Y	Y	Y
1	8.500000000	8.500000000	8.500000000	8.500000000		
2	9.366666667	9.233333333	9.266666667	9.300000000		
3	9.933333333	9.800000000	9.733333333	9.700000000		
4	11.566666670	10.400000000	10.133333330	9.966666667		
5	14.133333330	11.300000000	11.000000000	10.300000000		
6	17.066666670	12.800000000	11.900000000	10.633333330		
7	21.366666670	14.400000000	12.833333330	10.766666670		
8	24.766666670	15.900000000	14.000000000	11.166666670		
9	27.633333330	17.500000000	15.133333330	11.400000000		
10	30.333333330	19.700000000	16.233333330	11.600000000		
11	32.966666670	22.133333330	17.700000000	11.700000000		
12	34.966666670	24.566666670	19.000000000	12.066666670		
13	37.133333330	26.733333330	20.333333330	12.300000000		
14	38.900000000	29.100000000	21.733333330	12.500000000		

ANOVA results | Multiple comparisons

	Ordinary one-way ANOVA ANOVA results				
1	Table Analyzed	Onion length			
2	Data sets analyzed	A-D			
3					
4	**ANOVA summary**				
5	F	7.489			
6	P value	0.0003			
7	P value summary	***			
8	Significant diff. among means (P < 0.05)?	Yes			
9	R squared	0.3017			
10					
11	**Brown-Forsythe test**				
12	F (DFn, DFd)	17.31 (3, 52)			
13	P value	<0.0001			
14	P value summary	****			
15	Are SDs significantly different (P < 0.05)?	Yes			
16					
17	**Bartlett's test**				
18	Bartlett's statistic (corrected)	43.97			
19	P value	<0.0001			
20	P value summary	****			
21	Are SDs significantly different (P < 0.05)?	Yes			
22					
23	**ANOVA table**	**SS**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
24	Treatment (between columns)	1065	3	355.2	F (3, 52) = 7.489	P=0.0003
25	Residual (within columns)	2466	52	47.43		
26	Total	3532	55			

Note that long (>30 characters, or multiline) column (row) titles were shortened to A, B, C... (1, 2, 3...)

ANOVA results | Multiple comparisons

Ordinary one-way ANOVA
Multiple comparisons

1	Number of families	1							
2	Number of comparisons per family	6							
3	Alpha	0.05							
4									
5	**Tukey's multiple comparisons test**	**Mean Diff.**	**95.00% CI of diff.**	**Significant?**	**Summary**	**Adjusted P Value**			
6	Column A vs. Column B	6.183	-0.7252 to 13.09	No	ns	0.0946	A-B		
7	Column A vs. Column C	8.652	1.744 to 15.56	Yes	**	0.0086	A-C		
8	Column A vs. Column D	11.91	5.001 to 18.82	Yes	***	0.0002	A-D		
9	Column B vs. Column C	2.469	-4.439 to 9.378	No	ns	0.7788	B-C		
10	Column B vs. Column D	5.726	-1.182 to 12.63	No	ns	0.1368	B-D		
11	Column C vs. Column D	3.257	-3.651 to 10.17	No	ns	0.5976	C-D		
12									
13	**Test details**	**Mean 1**	**Mean 2**	**Mean Diff.**	**SE of diff.**	**n1**	**n2**	**q**	**DF**
14	Column A vs. Column B	22.76	16.58	6.183	2.603	14	14	3.359	52
15	Column A vs. Column C	22.76	14.11	8.652	2.603	14	14	4.701	52
16	Column A vs. Column D	22.76	10.85	11.91	2.603	14	14	6.471	52
17	Column B vs. Column C	16.58	14.11	2.469	2.603	14	14	1.341	52
18	Column B vs. Column D	16.58	10.85	5.726	2.603	14	14	3.111	52
19	Column C vs. Column D	14.11	10.85	3.257	2.603	14	14	1.770	52

Note that long (>30 characters, or multiline) column (row) titles were shortened to A, B, C... (1, 2, 3...)

2. Root length average data and one-way ANOVA and multiple comparison analysis from GraphPad

	Group A	Group B	Group C	Group D
	0mM NaCl (Average Root length in cm)	50mM NaCl (Average Root length in cm)	100mM NaCl (Average Root length in cm)	200mM NaCl (Average Root length in cm)
	Y	Y	Y	Y
1	1.666666667	2.200000000	2.733333333	1.333333333
2	1.966666667	2.300000000	2.900000000	1.400000000
3	3.566666667	2.400000000	2.933333333	1.466666667
4	4.966666667	3.066666667	2.966666667	1.500000000
5	6.566666667	3.666666667	3.133333333	1.533333333
6	8.033333333	4.433333333	3.300000000	1.533333333
7	8.666666667	4.966666667	3.433333333	1.533333333
8	9.400000000	5.266666667	3.666666667	1.533333333
9	9.866666667	5.633333333	3.900000000	1.533333333
10	10.600000000	5.966666667	4.200000000	1.533333333
11	10.966666670	6.233333333	4.333333333	1.566666667
12	11.066666670	6.433333333	4.466666667	1.566666667
13	11.166666670	6.633333333	4.533333333	1.566666667
14	11.200000000	6.933333333	4.533333333	1.566666667

ANOVA results | Multiple comparisons

Ordinary one-way ANOVA
ANOVA results

Table Analyzed	Root length				
Data sets analyzed	A-D				
ANOVA summary					
F	24.99				
P value	<0.0001				
P value summary	****				
Significant diff. among means (P < 0.05)?	Yes				
R squared	0.5904				
Brown-Forsythe test					
F (DFn, DFd)	12.40 (3, 52)				
P value	<0.0001				
P value summary	****				
Are SDs significantly different (P < 0.05)?	Yes				
Bartlett's test					
Bartlett's statistic (corrected)	100.9				
P value	<0.0001				
P value summary	****				
Are SDs significantly different (P < 0.05)?	Yes				
ANOVA table	**SS**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Treatment (between columns)	291.4	3	97.14	F (3, 52) = 24.99	P<0.0001
Residual (within columns)	202.2	52	3.888		
Total	493.6	55			

ANOVA results | Multiple comparisons

Ordinary one-way ANOVA
Multiple comparisons

Number of families	1							
Number of comparisons per family	6							
Alpha	0.05							
Tukey's multiple comparisons test	**Mean Diff.**	**95.00% CI of diff.**	**Significant?**	**Summary**	**Adjusted P Value**			
Column A vs. Column B	3.112	1.134 to 5.090	Yes	***	0.0006	A-B		
Column A vs. Column C	4.190	2.213 to 6.168	Yes	****	<0.0001	A-C		
Column A vs. Column D	6.324	4.346 to 8.302	Yes	****	<0.0001	A-D		
Column B vs. Column C	1.079	-0.8994 to 3.056	No	ns	0.4763	B-C		
Column B vs. Column D	3.212	1.234 to 5.190	Yes	***	0.0004	B-D		
Column C vs. Column D	2.133	0.1554 to 4.111	Yes	*	0.0298	C-D		
Test details	**Mean 1**	**Mean 2**	**Mean Diff.**	**SE of diff.**	**n1**	**n2**	**q**	**DF**
Column A vs. Column B	7.836	4.724	3.112	0.7452	14	14	5.905	52
Column A vs. Column C	7.836	3.645	4.190	0.7452	14	14	7.952	52
Column A vs. Column D	7.836	1.512	6.324	0.7452	14	14	12.00	52
Column B vs. Column C	4.724	3.645	1.079	0.7452	14	14	2.047	52
Column B vs. Column D	4.724	1.512	3.212	0.7452	14	14	6.095	52
Column C vs. Column D	3.645	1.512	2.133	0.7452	14	14	4.048	52