The Effect of Salt Concentration on the Germination of Soybeans

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Abstract. In order to solve the problem of crop growth difficulty in saline-alkali soil, it is meaningful to discuss how much salt affects the growth and germination of soybean. Zhonghuang 35 with low salt tolerance and Zheng 1311 with high salt tolerance were selected respectively, and NaCl solutions with concentrations of 0.250, 0.250, 0.125, 0.0625 and 0 mol L-1 were set for hydroponics, the effect of NaCl concentration on soybean germination was determined by variance analysis. The results showed that the concentration of NaCl was significantly correlated with the germination rate of Zhonghuang 35 and Zheng 1311 soybean varieties. The health of soybean is inversely proportional to NaCl concentration. The effect of NaCl concentration on germination in Zhonghuang 35 was more obvious than that in Zheng 1311. This study provides a theoretical basis for soybean growth in saline-alkali soil.

Keywords: Saline-alkali soil; salt concentration; crop growth; soybeans.

1. Introduction

Saline soil, soil that contains relatively high salt (NaCl) concentration, is widely recognized to be detrimental to plant growth, therefore few areas of saline land are reclaimed. However, some soybeans can tolerate the negative effect of salt on their germination and growth under certain concentrations. Since the composition of most saline soil is mainly sodium chloride, it is meaningful to discuss to what extent salt can affect the growth and germination of soybeans.

Zhonghuang 35 and Zheng 1311 are the beans being examined. A research conducted in the Chinese Academy of Science indicated their salt tolerance features: After emerging in 5 L of solution that contains 150 mmol L^{-1} of NaCl for 15 days, the results presented that Zhonghuang 35 is moderately salt tolerant during the emergence stage and highly sensitive to salt during the seeding stage, while Zheng 1311 is highly salt tolerant during the emergence stage and moderately sensitive to salt during the seeding stage (Liu et al., 2020).

The germination of seeds generally involves four processes: water absorption and swelling, sprouting, and germination. Oxygen, moisture, and temperature are the main conditions for germination. Additionally, soybean seeds typically germinate within 4-7 days of planting, and the process does not need any light. The conditions for germination is the same for Zhonghuang 35 and Zheng 1311 (Li et al., 2021).

This essay aimed to investigate how the concentration of sodium chloride (mol dm^{-3}) affects the germination and growth of soybeans. Zhonghuang 35, having a relatively low salt tolerance, and Zheng 1311, having a relatively higher salt tolerance are the types of soybeans used (Liu et al., 2020). The beans are selected to have a similar mass of 0.2±0.02 g. The result will be examined by Analysis of Variance (ANOVA) to determine the extent of effects of NaCl concentration on the germination of soybeans. I hypothesize that the degree of health of soybeans is inversely proportional to the concentration of NaCl, and the null hypothesis is that the degree of health of soybeans has no relationship with the concentration of NaCl.

2. Materials and methods

2.1 Experimental equipment

The equipment used in this experiment and its parameters are shown in Table 1.

Table 1 Experimental equipment

Items	Properties
Electronic scale x1	±0.001 g
Spoon x1	Made of plastic that do not react with sodium chloride
Tweezer x1	Made of plastic that do not react with sodium chloride, water, cellulose, and cell wall of beans.
Humidity meter and temperature meter x1	±0.2°C for temperature, ±2%RH for humidity.
Incubator with insulating layer x5	±0.2°C
250 cm^3 Beaker x25	-
50 cm^3 Measuring cylinder x5	±1 cm^3
25 cm^3 Measuring cylinder x1	±0.1 cm^3
Hydroponic medium	Water absorbance rate of 50%, mass: 5000 g. Made of cellulose that can not be absorbed by plants
NaCl powder	Humidity 0, purity 99.5%, molar mass: 58.44, mass: 27.394 g
Dropper x6	-
500 cm^3 Volumetric flask x1	±0.25 cm^3
0.45 g Zhonghuang 35 x125	±0.2 g

2.2 Experimental design

2.2.1 Sterilizing the apparatus

Firstly, use soap water to rinse out observable impurities on the spoon, tweezer, beakers, droppers, and volumetric flask. Secondly, use distilled water to rinse the apparatus until no presence of bubbles and water evenly adhere to the wall of the apparatus. Thirdly, place the apparatus aside until water is dried.

2.2.2 Choosing the beans and sterilizing the selected beans

Firstly, use an electronic scale to measure the mass of beans. Secondly, select 125 beans with a mass of 0.20±0.02 g and without obvious abnormal appearance. Thirdly, use distilled water to rinse out observable impurities on the surface of the selected beans. Fourthly, immerse the beans in 70% concentrated ethanol for 1 minute. Fifthly, use distilled water to rinse the beans 5 times.

2.2.3 Preparing saline medium

Firstly, using the measuring cylinder to measure 50cm^3 of distilled water and pour it into a 250 cm^3 beaker. Secondly, leave one beaker with only distilled water, and, measured by the electronic scale, add NaCl with a mass of 2.922, 1.461, 0.7305, and 0.3653 g respectively into the rest of the beakers, to get 5 samples of solution with a concentration of 0.500, 0.250, 0.125, 0.0625, and 0 mol L^{-1} respectively. Thirdly, pour the solution into the 5 measuring cylinders respectively and the volume by dropper to get an accurate figure. Fourthly, after NaCl is completely dissolved, add 100 g of the hydroponic medium into each beaker.

2.2.4 Setting apparatus

Firstly, install the incubator and set the humidity of 50% and temperature of 25C°. Secondly, put the 5 beans into the beakers. Thirdly, put the beakers into the incubator and close the incubator to prevent the entry of light because light can negatively affect the germination of soybeans and that is what the most Chinese farmers and researchers do (Wang, L., & Wang, J., 2015).

2.2.5 Germinating the beans

Firstly, record the ratio of germination every 12 hours. Secondly, remove the existing medium rinse the beakers, repeat step 3, and put them back into the incubator. Repeat the experiment 5 times for each type of the beans.

2.3 Experimental variable selection and the condition for the growth

2.3.1 Independent Variables

The concentration of NaCl. Five groups of beans are immersed in the solution where they have the concentration of 0.5, 0.25, 0.125, 0.0625, and 0 mol L^{-1} respectively.

2.3.2 Dependent Variables

The ratio and rate of germination are the dependent variables measured to determine the extent of the effect on the health of beans.

2.3.3 Controlled Variables

For each group of soybeans, the condition for germination is the same. The optimum conditions for the germination of soybeans include appropriate moisture, temperature, and pH. Precisely, soybean seeds should be germinated with a moisture content of 50-60%, a temperature of 25-30°C, and a pH of 6-7 (Zhou et al., 2021).

2.3.4 The condition for the growth

The condition for the growth is designed as followings: Temperature: the temperature in the incubator and solution is set to be 25C°; pH value: the pH value in the solution is set to be the neutral value of 7; Moisture: the humidity is set to 50%; Volume of NaCl solutions: hypertonic medium in each group is damped with 50 cm^3 of NaCl solution; Hydroponic medium: the cellulose hypertonic medium has a water absorption rate of 50%, and 100 g of hypertonic medium is placed in each sample; Type of bean. The beans are Zhonghuang 35 and Zheng 1311. Experiments for each type is conducted separately; Air: the concentration of each component in air in where the experiment is conducted is the same over the world, which consist of 78% of nitrogen, 20% of oxygen and 2% of other gases.

2.4 Safety and Environmental Considerations

Referencing human safety issues, there are only ethanol and micro-biotics on beans that might cause some accidents. For the direct potential damage of ethanol keeping in touch with a high concentration of ethanol solution for a long time. It is prevented by the isolation of goggles and gloves. In addition, preparing chemicals in a fume box where has great ventilation and isolation from fire and electricity is conducted to prevent explosions. For the micro-biotics on beans, face masks and gloves are worn to prevent potential affection, and sterilization is conducted every day. In addition, dead beans are picked, sterilized, and thrown into a specialized trash can for bio-hazards, then carried away by trash disposal companies. With regard to the environmental considerations, experimented beans and the saline solution needed to be treated. Hence, the beans are sent to the trash disposal companies by throwing the beans into the bio-hazards trash cans. And the saline water is directedly poured into the water sink, since the volume and concentration of the solution are low, which is not likely to cause any problems.

3. Results and discussion

3.1 Relationship between NaCl concentration and germination amount of soybean

Regarding the trends, for Zhonghuang 35, the period of time when the most soybeans germinated delayed slightly when the concentration of NaCl increased. Meanwhile, the rate of germination decreased about 20% when the concentration increased from 0.0625 to 0.125 mol L^{-1} and the trend of decreasing rose to about 50% when the concentration increased from 0.125 to 0.250 mol L^{-1} and

from 0.250 to 0.500 mol L^{-1}. This presented a fact that the range of being strongly salt tolerant of Zhonghuang 35 is from 0 to 0.0625 mol L^{-1}.

For Zheng 1311, the period of time when the most soybeans germinated did not delay when the concentration of NaCl increased, however, the rate of germination delayed significantly. Meanwhile, the rate of germination decreased about 20% when the concentration increased from 0.250 to 0.5 mol L^{-1}. This presented a fact that the range of being strongly salt tolerant of Zheng 1311 is from 0 to 0.250 mol L^{-1}.

Overall, there is only negative impact resulted by the increase in NaCl concentration, indicating that the degree of health of soybeans is inversely proportional to the concentration of NaCl, which proved my estimation.

Table 2 The results of the number of the soybeans germinated per 12hours

Zhonghuang 35 Germination Rate under NaCl Concentration: 0mol/L

	0h	12h	24h	36h	48h	60h	72h	84h	96h	108h	120h	132h	144h	156h	168h	170h	182h
Experiment 1	0	0	0	0	0	0	0	3	3	5	5	5	5	5	5	5	5
Experiment 2	0	0	0	0	0	0	0	2	4	4	4	4	4	4	4	4	4
Experiment 3	0	0	0	0	0	0	0	1	5	5	5	5	5	5	5	5	5
Experiment 4	0	0	0	0	0	0	2	3	5	5	5	5	5	5	5	5	5
Experiment 5	0	0	0	0	0	0	0	2	4	5	5	5	5	5	5	5	5

Zhonghuang 35 Germination Rate under NaCl Concentration: 0.0625mol/L

	0h	12h	24h	36h	48h	60h	72h	84h	96h	108h	120h	132h	144h	156h	168h	170h	182h
Experiment 1	0	0	0	0	0	0	1	3	5	5	5	5	5	5	5	5	5
Experiment 2	0	0	0	0	0	0	0	4	5	5	5	5	5	5	5	5	5
Experiment 3	0	0	0	0	0	0	0	4	5	5	5	5	5	5	5	5	5
Experiment 4	0	0	0	0	0	0	3	3	5	5	5	5	5	5	5	5	5
Experiment 5	0	0	0	0	0	0	0	1	4	4	4	4	4	4	4	4	4

Zhonghuang 35 Germination Rate under NaCl Concentration: 0.125mol/L

	0h	12h	24h	36h	48h	60h	72h	84h	96h	108h	120h	132h	144h	156h	168h	170h	182h
Experiment 1	0	0	0	0	0	0	0	2	4	4	4	4	4	4	4	4	4
Experiment 2	0	0	0	0	0	0	0	1	3	4	4	4	4	4	4	4	4
Experiment 3	0	0	0	0	0	0	0	3	3	3	3	3	3	3	3	3	3
Experiment 4	0	0	0	0	0	0	0	3	4	4	4	4	4	4	4	4	4
Experiment 5	0	0	0	0	0	0	0	1	3	3	3	3	3	3	3	3	3

Zhonghuang 35 Germination Rate under NaCl Concentration: 0.250mol/L

	0h	12h	24h	36h	48h	60h	72h	84h	96h	108h	120h	132h	144h	156h	168h	170h	182h
Experiment 1	0	0	0	0	0	0	0	0	1	1	2	2	2	2	2	2	2
Experiment 2	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2
Experiment 3	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1
Experiment 4	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
Experiment 5	0	0	0	0	0	0	0	1	3	3	3	3	3	3	3	3	3

Zhonghuang 35 Germination Rate under NaCl Concentration: 0.500mol/L

	0h	12h	24h	36h	48h	60h	72h	84h	96h	108h	120h	132h	144h	156h	168h	170h	182h
Experiment 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Experiment 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Experiment 3	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1
Experiment 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Experiment 5	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1

Zheng 1311 Germination Rate under NaCl Concentration: 0mol/L

	0h	12h	24h	36h	48h	60h	72h	84h	96h	108h	120h	132h	144h	156h	168h	170h	182h
Experiment 1	0	0	0	0	0	0	0	0	4	5	5	5	5	5	5	5	5
Experiment 2	0	0	0	0	0	0	0	0	4	5	5	5	5	5	5	5	5
Experiment 3	0	0	0	0	0	0	0	0	3	4	4	4	4	4	4	4	4
Experiment 4	0	0	0	0	0	0	0	0	5	5	5	5	5	5	5	5	5
Experiment 5	0	0	0	0	0	0	0	0	4	5	5	5	5	5	5	5	5

Zheng 1311 Germination Rate under NaCl Concentration: 0.0625mol/L

	0h	12h	24h	36h	48h	60h	72h	84h	96h	108h	120h	132h	144h	156h	168h	170h	182h
Experiment 1	0	0	0	0	0	0	0	0	5	5	5	5	5	5	5	5	5
Experiment 2	0	0	0	0	0	0	0	0	3	4	4	5	5	5	5	5	5
Experiment 3	0	0	0	0	0	0	0	0	4	5	5	5	5	5	5	5	5
Experiment 4	0	0	0	0	0	0	0	0	4	5	5	5	5	5	5	5	5
Experiment 5	0	0	0	0	0	0	0	0	4	4	5	5	5	5	5	5	5

Zheng 1311 Germination Rate under NaCl Concentration: 0.125mol/L

	0h	12h	24h	36h	48h	60h	72h	84h	96h	108h	120h	132h	144h	156h	168h	170h	182h
Experiment 1	0	0	0	0	0	0	0	0	4	4	5	5	5	5	5	5	5
Experiment 2	0	0	0	0	0	0	0	0	3	3	4	5	5	5	5	5	5
Experiment 3	0	0	0	0	0	0	0	0	5	5	5	5	5	5	5	5	5
Experiment 4	0	0	0	0	0	0	0	0	2	4	4	5	5	5	5	5	5
Experiment 5	0	0	0	0	0	0	0	0	4	4	4	4	4	4	4	4	4

Zheng 1311 Germination Rate under NaCl Concentration: 0.250mol/L

	0h	12h	24h	36h	48h	60h	72h	84h	96h	108h	120h	132h	144h	156h	168h	170h	182h
Experiment 1	0	0	0	0	0	0	0	0	4	4	5	5	5	5	5	5	5
Experiment 2	0	0	0	0	0	0	0	0	3	5	5	5	5	5	5	5	5
Experiment 3	0	0	0	0	0	0	0	0	3	3	4	5	5	5	5	5	5
Experiment 4	0	0	0	0	0	0	0	0	0	3	3	4	4	4	4	4	4
Experiment 5	0	0	0	0	0	0	0	0	2	4	4	5	5	5	5	5	5

Zheng 1311 Germination Rate under NaCl Concentration: 0.500mol/L

	0h	12h	24h	36h	48h	60h	72h	84h	96h	108h	120h	132h	144h	156h	168h	170h	182h
Experiment 1	0	0	0	0	0	0	0	0	2	2	2	3	3	3	3	3	3
Experiment 2	0	0	0	0	0	0	0	0	3	3	5	5	5	5	5	5	5
Experiment 3	0	0	0	0	0	0	0	0	1	1	3	4	4	4	4	4	4
Experiment 4	0	0	0	0	0	0	0	0	0	3	4	4	4	4	4	4	4
Experiment 5	0	0	0	0	0	0	0	0	2	2	3	3	3	3	3	3	3

Note: The results are recorded at 8 a.m. and 8 p.m. every day

3.2 Statistical analysis of ANOVA

In order to conduct an One-way analysis of variance (ANOVA), the data should first be selected. The data recorded when the number of germinated soybeans stayed the same (when all seeds are ungerminated and when all seeds are germinated) is excluded. Therefore, the selected data of Zhonghuang 35 is within the range of 72h ≤ time ≤ 132h, while for Zheng 1311 is within the range of 96h ≤ time ≤ 132h.

Table 3 the number of beans germinated within the range and under the NaCl concentration of 0, 0.500, 0.250, 0.125, 0.0625 mol L-1 respectively for Zhonghuang 35

0.4	2.2	4.2	4.8	4.8	4.8
0.8	3	4.8	4.8	4.8	4.8
0	2	3.4	3.4	3.4	3.4
0	0.2	1.4	1.6	1.8	1.8
0	0	0.4	0.4	0.4	0.4

Table 4 the number of beans germinated within the range and under the NaCl concentration of 0, 0.500, 0.250, 0.125, 0.0625 mol L-1 respectively for Zheng 1311

4	4.8	4.8	5
4	4.6	4.8	5
3.6	4	4.4	4.8
2.4	3.8	4.2	4.8
1.6	2.2	3.4	3.8

Table 5 The ANOVA results for Zhonghuang 35

Data Summary				
Groups	N	Mean	Std. Dev.	Std. Error
Group 1	6	3.5333	1.8359	0.7495
Group 2	6	3.8333	1.6513	0.6741
Group 3	6	2.6	1.3914	0.568
Group 4	6	1.1333	0.8165	0.3333
Group 5	6	0.2667	0.2066	0.0843

ANOVA Summary					
Source	Degrees of Freedom DF	Sum of Squares SS	Mean Square MS	F-Stat	P-Value
Between Groups	4	56.7239	14.181	8.1102	0.0002
Within Groups	25	43.7134	1.7485		
Total:	29	100.4372			

Table 6 The ANOVA results for Zheng 1311

Data Summary				
Groups	N	Mean	Std. Dev.	Std. Error
Group 1	4	4.65	0.4435	0.2217
Group 2	4	4.6	0.432	0.216
Group 3	4	4.2	0.5164	0.2582
Group 4	4	3.8	1.0198	0.5099
Group 5	4	2.75	1.0247	0.5123

ANOVA Summary					
Source	Degrees of Freedom DF	Sum of Squares SS	Mean Square MS	F-Stat	P-Value
Between Groups	4	9.7	2.425	4.4252	0.0146
Within Groups	15	8.22	0.548		
Total:	19	17.92			

For the Zhonghuang 35 variety, the ANOVA results show an F-statistic of 8.1102 and a P-value of 0.0002. With a P-value less than 0.05, we can reject the null hypothesis, which states that there is no relationship between the degree of health of soybeans and the concentration of NaCl. This suggests that the germination of Zhonghuang 35 is significantly affected by NaCl concentration, supporting the alternative hypothesis that the degree of health of soybeans is inversely proportional to the concentration of NaCl.

Similarly, for the Zheng 1311 variety, the ANOVA results reveal an F-statistic of 4.452 and a P-value of 0.0146. Since the P-value is also less than 0.05, we can reject the null hypothesis for this soybean variety as well. This indicates that the germination of Zheng 1311 is also significantly influenced by NaCl concentration, albeit with a lower F-statistic compared to Zhonghuang 35.

3.3 Test reliability and practical value

One strength is that there is a relatively low amount of systemic errors. For instance, the most of influential factors to the growth of seeds are considered and applied to be standard or widely recognized values and are kept throughout the experiment. Another strength is that the experiment is designed to be as simplified but reliable as it can be under my current academic knowledge. For example, I did not use dirt and used hydroponic medium instead, which avoided the interruption to the experiments' results due to the various uncertain impurities contained in the dirt; Hence, the accuracy and reliability of the result increased.

Of course, this experiment still has some shortcomings. One weakness is that a limited types of soybeans are tested. Different types of beans have different extents of salt tolerance. Therefore, the extent of the effect of salt concentration on the germination of beans measured is likely to be only referenced for the Zhonghuang and Zheng series. Another weakness is that the results obtained in this experiment are relatively not worth referencing in the practice. There are numbers of influences that I did not consider in my experiments, such as insects and different types of soil presented all over the area where can plant soybeans; these unconsidered factors are also quite important in practice, hence the value of this experiment might be lowered.

As to applications, although limited amount types of soybeans are examined, the results and conclusion for the two specific beans, Zhonghuang 35 and Zheng 1311, can still be worth referencing if farmers are deciding to plant them on the saline soils.

For the extension, an extension could be made by keeping on tracking the effect of NaCl on the soybeans to the stage of further growth and blossom, which can make this research more applicable in agriculture on the saline soils. In addition, other types of beans, can be used in the experiment, which might present slightly different results and hence make the experiment to be more worth referencing.

4. Conclusions

In conclusion, the data analysis shows that both Zhonghuang 35 and Zheng 1311 soybean varieties exhibit a significant relationship between NaCl concentration and germination. This supports the initial hypothesis that the degree of health of soybeans is inversely proportional to the concentration of NaCl. However, it is important to note that the effect of NaCl concentration on germination is more pronounced in the Zhonghuang 35 variety compared to the Zheng 1311 variety. This is in line with the prior research by Liu et al. (2020), which indicated that Zhonghuang 35 has a relatively lower salt tolerance compared to Zheng 1311.

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