James Fruen

The Effect Of Sodium Chloride Concentration In Growing Medium On the Growth And Total Germination Of Cress Seeds

Scenario

There are a number of things that can influence how/if a plant grows and germinate. These include;

- Amount and intensity of sunlight.
- CO^2 concentration of the air.
- Temperature and humidity of the atmosphere.
- PH of the water and soil.
- Salt concentration of the soil and water.
- Quality of the soil, i.e. Minerals and nutrients.
- Use of fertilisers and pesticides.

The dependant variable (DV) in this scenario is the germination and growth rate of the seeds. The factors that effected the growth and germination of the seeds where therefore the independent variables (IV).

Introduction

Germination is triggered by the uptake of water into the seed, water always moves from a place of higher water potential to a place of lower water potential. Water potential is a measure of the tendency of water to move from high free energy to lower free energy. Distilled water in an open beaker has a water potential of 0 (zero), the highest water potential possible. The addition of solute decreases water potential. In cells, water moves by osmosis to areas where water potential is lower. To release the food stored in seeds that help aid the plant in its early growth, they must first hydrolysed. Hydrolysis is a chemical decomposition process that uses water to split chemical bonds of substances. There are two types of hydrolysis; acid and enzymatic. Food stores are appropriate for enzymatic hydrolysis because they contain cellulose.

The first stage of germination is imbibition. This is where water is brought into the seed via hydrophilic molecules. This water acts as a carrier for the hormone Gibberellic Acid; this hormone is transported to the endosperm where much of the protein is stored. Here the water activates proteolytic enzymes that break down the protein stores into amino acids, used for growth. The hormone activates the gene in the plants DNA that codes for amylase. This gene then transcribes to mRNA, which in turn translates to for the enzyme amylase. Amylase hydrolyses the starch present in storage to produce maltose. This carbohydrate is used as the respiratory substrate. Respiration provides the energy required to allow the plant to make new polypeptides and therefore grow. Starch is insoluble and so does not affect the water potential.

If sodium chloride concentration is too high around the seed exterior, the water potential around the seed will be lower than the water potential inside the seed. This means that no water will enter the seed, preventing germination from starting and removing any trace amount of water inside the seed via osmosis. As the water potential around the seed increases, water will be taken into the seed and trigger germination.
The statistical test used will be the Chi-Square Test. This is because the variation between the results gathered, and the results expected, needs to be measured and recorded, then checked against the published data table to see whether the result is within the 5% significant difference range.

Other Controlled Variables.

The factors affecting this experiment that will need to be controlled are: -

- **Seed Size** – All the seeds will be the same species and so should have a similar shape and size.
- **Seed Age** – All seeds will be taken from the same packet.
- **Seed Species** – All seeds will be cress seeds.
- **Time** – All seeds will be left for the same period of time
- **Amount Of Water** – All seeds will be given a set $15cm^3$ of water. If more water is required during the experiment, the same will be added to every tray.
- **Spacing** – Each seed will be placed in a square matrix 2cm away from other seeds.
- **Temperature** – The seeds will all be exposed to room temperature.
- **Light Intensity** – All seeds will be placed in front of the same window.
- **Nutrients** – Seeds will be grown on filter paper sitting on cotton wool.
- **PH** – A neutral PH cotton wool and filter paper will be used. NaCl is also neutral.

Null Hypothesis
The change in sodium chloride concentration will have no effect on the total germination or growth rate of the seeds. Any experimental discrepancy will be due to chance.

Safety
Wear safety glasses at all times to prevent salt water coming in contact with eyes.

Preliminary Experiment

To determine the concentration ranges that will give the best results a preliminary experiment is needed. This will determine the sodium the sodium chloride percentage that stop all seeds germinating, as there is no point trying any concentration greater then this.

The method will be identical to the main experiment but the concentrations used will be 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0.

Apparatus

6 Trays	36 Test Tubes
Bag Of Cotton Wool	Test Tube Holders
Bottled Distilled Water	2 $15cm^3$ Bulb Pipette
120 Cress Seeds	Clingfilm
Sodium Chloride	$150cm^3$ Beaker

12cm By 16cm Pieces Of Filter Paper

Method

1. Line trays with the cotton wool
2. Place the filter over the top
3. Make a serial dilution starting from 2% concentration

Serial Dilution

1. *Label five test tubes 1-5*
2. *In the beaker add 2g of sodium chloride to $98cm^2$ of distilled water*
3. *Using the bulb pipette place $15cm^3$ of the solution in test tubes 1 and 2*
4. *Using the other bulb pipette add $15cm^3$ of distilled water to test tube 2*
5. *Shake test tube 2 until mixed, then remove $15cm^3$ of the solution from tube 2 and place it in tube 3*
6. *Repeat this process for sequential test tubes so each is half the concentration of the previous test tube.*
7. *Discard the $15cm^3$ taken from test tube 5*

Method Continued…

4. Create an experimental control by labelling a test tube '6' and placing $15cm^3$ of distilled water in it.
5. Mark the 6 trays with the concentration range (ie. 2.0 – 0.0)
6. Put the relevant solution into the cotton wool lined trays
7. Place the 20 seeds in each tray, remembering to leave 2cm gap between the seeds on each side.
8. Cover the trays with the clingfilm and leave on a windowsill
9. Take the measurements at the set times
10. Repeat the experiment 5 times
11. If the cotton wool dries out, the same amount of water should be added to each tray

Sample Tables

		Mean No. Of Seeds Germinated After (Days)							
Concentration (%)	**Repeat**		**2**	**4**	**6**	**8**	**10**	**12**	**14**
2	1								
	2								
	3								
	4								
	5								
	6								
1	1								
	2								
	3								
	4								
	5								
	6								
0.5	1								
	2								
	3								
	4								
	5								
	6								
0.25	1								
	2								
	3								
	4								

	5								
	6								
0.125	1								
	2								
	3								
	4								
	5								
	6								
0	1								
	2								
	3								
	4								
	5								
	6								

Length Of Radicle (cm) After (Days)

			2	4	6	8	10	12	14
Conc. (%)	**Repeat**								
2	1								
	2								
	3								
	4								
	5								
	6								
	Mean								
1	1								
	2								
	3								
	4								
	5								
	6								
	Mean								

0.5	1								
	2								
	3								
	4								
	5								
	6								
	Mean								
0.25	1								
	2								
	3								
	4								
	5								
	6								
	Mean								
0.125	1								
	2								
	3								
	4								
	5								
	6								
	Mean								
0	1								
	2								
	3								
	4								
	5								
	6								
	Mean								

Statistical Test

This set of data is most suited to a Chi-Squared test. For the total germination after 14 days of each result, the following equation should be carried out. The expected result is the values of 0% concentration results.

$$X^2 = \mathbf{\Sigma} \frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}}$$

Sample Chi-Square Table

	Concentration				
	2.000	1.000	0.500	0.250	0.125
Obs-Exp					
$(\text{Obs-Exp})^2$					
$\frac{(\text{Obs-Exp})^2}{\text{Exp}}$					

Total =

There are four degrees of freedom.

Ananlysis

Chi-Square Test

The statistical test used will be a chi-square test. This is because the variation between the results gathered and the expected results need to be measured to check if there is a significant difference. Then the results will be checked against the published data table to see whether the result is within the 5% significant difference range.

		Concentration (%)		
	1.000	0.500	0.250	0.125
Obs-Exp	0.7	0.3	0.5	0.3
$(Obs-Exp)^2$	0.49	0.09	0.25	0.09
$\frac{(Obs-Exp)^2}{Exp}$	0.0408	0.0075	0.0208	0.0081

Demonstration for 1.000%

1) $12.0 - 11.7 = 0.70$
2) $0.07^2 = 0.49$
3) $0.49 / 12 = 0.0408$

$X^2 = 0.0767$ (3 s.f.)

There are 4 degrees of freedom.

The probability of the change being due to chance is greater than 0.99 (99%) so it is highly unlikely that NaCl concentration had any effect on the germination of seeds after two days.

Null Hypothesis

This was that the change in sodium chloride concentration would have no effect on the total germination of the seeds, and that any experimental discrepancy will be due to chance.

The results clearly show that this is the case, and that the NaCl concentration of the growing medium had no effect on likelihood of a seed germinating.

Conclusion

The overall pattern shown in each of the three time measurements is that as the concentration of NaCl increases the amount of growth by the seed decreases.

After two days, the difference in growth and general health is visible; the straight line on the graph also shows this. Between the fifth and seventh day the graph starts to show more detail as to the differences in growth between the different concentrations. It shows that the growth is much faster at lower concentrations, and much slower at higher concentrations of sodium chloride. This is due to the amount of water taken in to the root via osmosis. In pure water the rate of water uptake is greatest and fastest and therefore so is growth; as relefted by the graphs. At higher concentrations of NaCl (sodium chloride) the water potential is much less, and so less water is taken up via osmosis and more slowly. Having less water available limits the amount of ATP produced by the plant, which means the plant grows less.
The difference in water potential is less important at the beginning of growth as imbibition is due to hydrophilic molecules, and so changing the water potential around the seed with have very little effect on early growth. However as soon as the seed produces a shoot and starts photosynthesising, water will be taken in via the root and will be greatly affected by water potential. This is why the line drawn for day two has points closer together that the other two. The Chi-Square test shows that the germination of the seeds is not dependant on the concentration of NaCl in the surrounding water. This is due to imbibition being started by hydrophilic colloid molecules rather then via osmosis.

Evaluation

During the experiment I came across no odd results. All measurements where close enough to the mean data to be reliable. This was due to the nature of the way the data was collected. Any odd results in individual experiments where cancelled out by the 71 other seeds (in repeat experiments)
The method as a whole provided reasonably reliable results although there are some improvements that could be made.
The first source of error is due to the equipment used. To make each new dilution in the serial dilution, a measuring cylinder was used which is only accurate to $\pm 0.5 cm^3$. This could have been improved by using a much more accurate piece of equipment like a pipette. The second source of error could have been in using rulers that are accurate to ±0.05cm, luckily this was less of a problem because readings where only taken to one decimal place.
The third source of error could have been in collecting repeat data from other students in the class. Each student has a slightly different way of carrying out the measurements of the radical etc. However, this problem is also minor and will have minimal affect on the results. In contrast, a major problem is the time intervals between measuring the radical lengths. There where to few results collected to make an accurate statement about the growth rate or germination,

only a general pattern can be made from the results gathered in this experiment, as there are only 3 different intervals for measuring the growth. To make the experiment more accurate, measurements need to be taken at much more closer intervals (eg every few hours for the first few days) then daily after that. The results produced from an experiment with that sort of structure, would yield much more conclusive results as to rate of growth. Finally, again to do with the serial dilution technique used, solutions should have been prepared up from 0.1% to 1.0% in increments of 0.1%. This again would yield a much more accurate representation of each concentration being used, and maybe smooth out overall trends in the results.