Biology

How does the concentration of Ca^{2+} at 0.650M, 1.30M, 1.95M, and 2.60M affect the stem growth height of garlic (*Allium sativum)* cloves?

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Introduction

When I was young, I learned that calcium was extremely important in humans, as it assisted in the development of healthy bones, as well as blood clotting. However, calcium was never mentioned when I learnt about plants, so I wondered how calcium affected the development of plants. I decided to choose garlic (Allium sativum) because they could be grown in close proximity in a hydroponic setup, allowing for better absorption of the calcium. Furthermore, I also thought that because there were many cloves of garlic in a bulb, there would be less variation that could alter how the plant responded to the calcium.

Calcium is classified as a secondary macronutrient, meaning that while it is not as crucial as primary macronutrients like nitrogen for the growth of plants, it is still very important for the healthy growth of a plant. It plays a structural role in plant cells and membranes, maintaining a sturdy foundation for the growth of these structures (White, 2003). In garlic, calcium is typically the highest when garlic undergoes bulb formation, as it ensures that the bulb develops properly. As well, it acts as a counter cation for anions in the central vacuole, and also works as an intracellular messenger in the cytosol of plant cells (Marschner, 1995). Calcium is taken in from the soil through the roots, and is delivered to the rest of the plant through the xylem (White, 2003). Calcium passes through the roots through the cytoplasm of cells that are connected by plasmodesmata, pores of channels in between plant cells. It can also pass through the spaces outside the cytoplasm of the plant cells, known as the apoplast (White, 2003). After passing through the xylem, the calcium enters the plant cells through Ca^{2+}-permeable channels.

Calcium deficiency is often rare in nature, but can be found in highly acidic soils. Symptoms of calcium deficiency are typically found in young leaves, where transpiration is low due to the size, as well as in enclosed tissues. Tipburn, the browning of leaf edges, can occur in younger leaves. Fruits, which are typically fed by the phloem can also exhibit calcium deficiency, demonstrated by the 'bitter pit' in calcium deficient apples. (White, 2003). In garlic, calcium deficiency can result in poor root growth, as root hairs do not form, affecting the uptake of water and other nutrients.

However, excess calcium can also affect plants. Calcium toxicity can prevent seed germination, as well as reduce growth rates and heights.

Hypothesis

Null hypothesis (H_0): As Ca^{2+} concentration increases, there will be no difference in the heights of the garlic stems.

Alternative Hypothesis (H_a): As Ca^{2+} concentration increases, the height of garlic stem growth will increase.

Variables

Independent variable: Calcium concentration (0.650M Ca^{2+}, 1.30M Ca^{2+}, 1.95M Ca^{2+}, 2.60M Ca^{2+} in supplement form)

Dependent variable: Garlic stem growth height (cm) (±0.1cm)

Controlled variables:

- Temperature - All cloves of garlic were placed on the same tray in the same spot, ensuring that the cloves are in the same temperature. Temperature measured during the day was around 20ºC and temperature during the night was 15ºC.
- Sunlight - All cloves of garlic were placed on the same tray. This meant that all cloves would receive the same amount of light per day.
- Source of Calcium - All of the calcium used was from calcium supplements, which came in pills. This ensured that there would be the same amount of calcium from the same source per pill.
- Type of container used - The containers that were used were all cut to the same height, made of the same plastic, and were the same size. This ensured that the containers would not affect how the garlic stems grew.
- Volume of water initially - The volume of water was initially 10mL. As well, the same amount of water was added to the containers every week. This ensured that the garlic would not become dehydrated.
- Type of garlic used - All the garlic was harvested from the same region and purchased at the Real Canadian Superstore

Materials

- 100 cloves of garlic
- 50 calcium supplements (contains a mixture of calcium carbonate, calcium citrate, calcium malate, calcium fumarate, calcium succinate)
- 1 stirring rod
- 25 small containers (approx. 40 mL each)
- 250 mL of tap water
- 1 large baking tray

Procedure

1. Place the 25 small plastic containers in a 5 container by 5 container square on the baking tray.
2. Fill each container with 10 mL of water
3. Place one tablet of calcium into the first column, place two tablets into the second column, place three tablets into the third column, and place four tablets into the fourth column. Leave one column with only water, this will be the control.
4. Stir all containers well, ensuring that the calcium is well dissolved.
5. Place four garlic cloves of similar size into each container. Ensure that the cloves are packed together tightly and are upright, with the tip of each clove pointing upwards, for optimal stem growth.
6. Measure the initial stem height for all the garlic cloves, and record it. Measure from the top of the garlic clove.
7. Record the qualitative observations of each of the garlic columns with each amount of calcium concentrations. As well, observe the root growth of the garlic cloves. 8. Repeat steps 6 and 7 once per week for three weeks.
8. Add 6 mL of water to each container after every time the garlic stem height is measured. 10. After measuring for the third week, dispose of the apparatus. Place the garlic in the compost and dispose of the water down the drain.

Risk Assessment

There are no significant ethical, environment, or health and safety risks associated with this experiment. The garlic and water used for this experiment should not be ingested. The garlic should be put into the compost or garbage, and the water can be disposed of down the drain. However, if there is excess calcium residue in the water then the residue can be disposed of in the garbage.

In this experiment, 1 calcium supplement represents 0.650M Ca^{2+}, 2 calcium supplements represents 1.30M Ca^{2+}, 3 calcium supplements represents 1.95M Ca^{2+}, and 4 calcium supplements represents 2.60M.

Processed Data

Table 2: Average garlic stem growth height for 5 trials, overall garlic stem growth height, standard deviation, and margin of error at 95% confidence for garlic cloves grown in a solution with no calcium supplements

	Average Stem Growth Height (cm)					Overall average stem growth height (cm)	Standard deviation (cm)	Margin of error (cm)
Week	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5			
0	0.25	0.08	0.08	0.13	0.13	0.13	0.062	± 0.122
1	8.43	5.65	9.78	0.63	7.15	6.33	3.16	±2.770
2	23.75	18.83	23.85	5.48		18.73	6.872	±6.02
3	34.43	29.75	36.98	18.95	36.43	31.31	6.681	±5.856

https://docs.google.com/spreadsheets/d/1vtMMOIqpAPl23Zl7OFFolh0z-eKkiFsFNoCQCf_co_4/edit?fbclid=IwAR20hi44KG2TqYFt7-pHCuxCk-icnqKpm9ctUymBpUYIWgEXVxYiApr8xSA#gid=0

(The Processed Data is available at this link on Sheet 1)

Margin of error for week 0 of garlic grown with no calcium supplements at a 95% confidence interval: (Standard deviation x 1.960) = 0.122 cm
The error bars in this graph indicate the margin of error at 95% confidence for the average heights of the garlic stems at various concentrations. The R^2 values demonstrate that there is a strong positive association between the points and the trend line.

Statistical Analysis

Table 3: One-way analysis of variance test for all groups of garlic

This is available on the Data sheet on Sheet 4.

Table 4: One-way analysis of variance test for no calcium supplements and one calcium supplement

This is available on the Data sheet on Sheet 5.

Table 5: One-way analysis of variance test for no calcium supplements and two calcium supplements

This is available on the Data sheet on Sheet 6.

Table 6: One-way analysis of variance test for no calcium supplements and three calcium supplements

This is available on the Data sheet on Sheet 7.

Table 7: One-way analysis of variance test for no calcium supplements and four calcium supplements
This is available on the Data sheet on Sheet 8.

It can be observed that for all of the Analysis of Variance tests, the p-value was greater than 0.05, indicating that there was not much statistical significance in the tests. As a result, the null hypothesis H_0 can't be rejected. Furthermore, the alternative hypothesis must be rejected, as there is still a correlation between the amount of calcium supplements and the growth height of the garlic stem.

Qualitative observations

Table 8: Garlic stem growth quality and garlic clove root growth quality over three weeks in solutions with different amounts of calcium

	Amount of calcium supplements				
Week	0	1	2	3	4
0	small shoots poking out of some garlic cloves, light green stems, clear water, no root growth	small stems growing, light green, somewhat translucent water, no root growth	small stems growing, light green, somewhat translucent water, no root growth	small stems growing, translucent water, no root growth	small stems growing, light green stems, translucent water, no root growth
1	Stems growing are darker green, some roots,	Stems are growing, new shoots sprouting from older stems, some roots	Medium green stems, Short roots	Light green stems, short roots, translucent water	Light green, short stems, somewhat short roots. Some calcium on roots
2	Darker tall green stems, some stems begin to separate, transparent water, some root growth	Dark and tall green stems, some separation in stems, some root growth, nearly transparent water	Shorter, dark green stems, some separation in stem growth, somewhat translucent water, some calcium on roots	Dark green stems, translucent water, some calcium on roots, separation in stems	Very dark green stems, separation in stems, many roots, calcium on roots, translucent water

3	Light green stem base, darker tip, transparent water, 2 shoots sprouting from same stem, some root growth	Green stem base, darker tip, transparent water, some root growth, 2 shoots sprouting from the same stem,	Light green stem base, green tip, somewhat transparent water, more and thicker root growth	Light green stem base, darker green tip, translucent water, some calcium on roots, thick root growth	Light green stem base, darker green tip, translucent water, thick and abundant root growth

Conclusion

The purpose of this experiment was to determine how the concentrations of Ca^{2+} at 0.650M, 1.30M, 1.95M, and 2.60M would affect the growth heights of garlic stems. From Tables 3-7, a p-value that is greater than 0.05 is observed for all Analysis of Variance tests, meaning that H_0 can't be rejected. H_a, the alternative hypothesis, must also be rejected, as it does not accurately reflect the results of this experiment. Instead, Table 2, Appendix B and Graph 1 demonstrate that garlic growing with 1 calcium supplement exhibits the greatest stem growth height, at 32.53 cm after 3 weeks, and that garlic growth decreases as more calcium supplements are added, which is seen by the final heights of 24.13 cm, 22.73 cm, and 21.56 cm for two, three, and four calcium supplements respectively. With 2 or more calcium supplements, garlic growth is significantly inhibited. This may be attributed to the inability of the garlic to take up other nutrients, as well as water, since there may be too much calcium that is surrounding the roots (White, 2003). Calcium's importance in developing the lamellar, or membrane, systems (Burstrom, 1968) was recorded in Table 8, which demonstrated that in general, higher concentrations of calcium led to thicker and more roots, as well as greener stems. Furthermore, over three weeks, the garlic stems with no calcium supplements demonstrated less root growth, which may be attributed to less mitochondrial function due to calcium deficiency (Florell, 1956). Graph 1 also demonstrates how 1 calcium supplement allowed for maximum growth, whereas no calcium supplements had a lower average height over the 3 weeks. Excessive calcium may also result in over-rigidity of the cell wall, further inhibiting plant growth. One major problem with this is that as the plant cell itself changes as the central vacuole swells and contracts, there must be some flexibility in the cell wall to allow for the changes in size. Furthermore, an over rigid cell wall results in the inability for the cells to grow as much, due to the cell wall limiting the size that the cell can reach. This is demonstrated in Graph 1 and Table B4, where the growth height of garlic stems decreases as the concentration of Ca^{2+}increases. This is most notable when the concentration of $Ca^{2+}$is at 2.60M but the average height of the garlic stems was only 21.56cm. Potential reasons for the extremely high p-values are discussed in the weaknesses.

Strengths

One of the strengths of this experiment was that the apparatus the garlic stems were grown in ensured that the growth of stems would promote more stem growth instead of devoting resources to growth of bulbs. As a result, the effectiveness of the different calcium concentrations could be better measured. Another strength of this experiment was that by growing the garlic in water only, it was easier to observe how calcium affected the growth of roots by removing the issue of having to remove the garlic from soil in order to observe the roots. As well, the small containers that were used made it so that, as opposed to planting the
garlic in a larger container, there would be less space for the garlic roots to grow, optimizing the uptake of calcium as opposed to other nutrients that may have been present in soil.

Extensions

One possible extension of this experiment could be to measure the growth of the garlic bulb in different soil calcium concentrations. This experiment can be conducted in both extremely low soil calcium concentrations, and extremely high calcium concentrations to explore the effects of absence of calcium and calcium toxicity respectively on garlic growth. This experiment can also focus on more qualitative observations by preparing microscope slides to study exactly how garlic cells are affected by the varying calcium concentrations. One further investigation could be to study how calcium concentrations affect soil microfauna. These tests can examine the effects of calcium toxicity and calcium deficiency on these microorganisms.

Weaknesses and Improvements

Weakness	How it affects data	Improvement
Size of garlic cloves	Larger garlic cloves (obtained from the outside of the bulb) may result in longer stems, as opposed to the smaller garlic cloves obtained from inside the bulb.	Ensure that all garlic cloves are roughly the same size, as it may result in more uniformity in data. Furthermore, the garlic could be obtained from the same batch, resulting in less differences in stem growth that may be based in genetics.

Organization of containers	In order to decrease the area that the cloves were spread across and to increase the uniformity of the data, the cloves were organized in a 5 by 5 square. However, as the stems grew in height, this meant that some of the cloves that were in the middle of the square may have been shielded by the cloves on the outside, resulting in less sunlight received and less growth.	Position the tray in a space where more direct sunlight can be received by the middle cloves, ie. positioning the light source above the cloves instead of at an angle.
Length of time the experiment was carried over	Since it generally takes over nine months for garlic to fully mature, a period of three weeks may be too short to calculate the true effects of calcium on the growth of garlic, even just the stem.	Carry the experiment over a longer period of time, such as two months, to truly understand the effects of calcium on garlic stem growth.

For references, footnotes and endnotes, click here.