

THE EFFECT OF DIFFERENT ASCORBIC ACID CONCENTRATIONS ON THE GROWTH OF *ALLIUM FISTULOSUM*

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Abstract

Ascorbic acid, also known as Vitamin C, is an essential micronutrient for the metabolism of almost all living organisms and plays a role in various physiological processes. It is synthesized by some animals and plants, and increasing its concentration in crops may have beneficial effects on human health. In plants, it plays a significant role in elongation, differentiation of cells, and programmed cell death. The meristem tissue in *Allium fistulosum* is responsible for rapid cell division and regeneration, determining plant morphology and growth rate. This research aims to investigate if different concentrations of ascorbic acid in solutions can increase the growth rate of green onions by helping stem cells differentiate and regrow the part of the plant that was removed. In the experiment, *Allium fistulosum* samples were exposed to different ascorbic acid concentrations, and the changes in root length and leaves height were measured over 14 days. The results of ANOVA and R^2 tests show that the samples with the highest concentration of vitamin C expressed the biggest growth, both in root length and in leaf height, while those with the lowest concentration expressed the opposite effect, having the smallest growth.

Keywords: Ascorbic Acid, Vitamin C, *Allium fistulosum*, Green Onions

1. Introdução

Ascorbic acid, commonly known as Vitamin C, is an essential micronutrient and a key element for the metabolism of almost all living organisms. The biological roles of vitamin C derive from its ability to function as a reducing agent, as it acts as an effective antioxidant as well as being required for preventing the over-oxidation of iron. It is a water-soluble compound that is involved in controlling reactive oxygen and the associated signaling events required for acclimation to environmental stress (SMIRNOFF, 2011).

Ascorbic acid is synthesized by some animals and plants, and it is considered a vitamin only for a few vertebrate species, including humans, that are unable

to synthesize it. In humans, vitamin C takes part in several physiological processes, such as iron absorption, collagen synthesis, immune stimulation, and epigenetic regulation (PACIOLLA et al, 2019). For this reason, increasing the concentration of ascorbic acid in crops may have beneficial effects on human health.

In plants, ascorbic acid takes part in the process of elongation, differentiation of cells and programmed cell death (PCD) (PACIOLLA et al, 2019). It also plays a significant role in increasing and controlling cell division, as it is required for the development of G1 and G2 phases (LISO, 1984).

The meristem is a type of tissue found in the buds and growing tips of the roots, that is responsible for

storing stem cells. Therefore, the activity of meristems determines plant morphology and growth rate. In *Allium fistulosum*, commonly known as green onions, the meristem tissue is located at the base of the vegetable, near the roots. This species was chosen for my investigation due to its capacity of rapid cell division and regeneration, which decreases the time needed for the experiment (Daigen et al 2001). Due to the many stem cells present in the meristem, they will be able to divide and differentiate themselves into specialized cells capable of performing different functions, quickly regenerating an entire new structure for the plant and completely replacing the one that has been cut off.

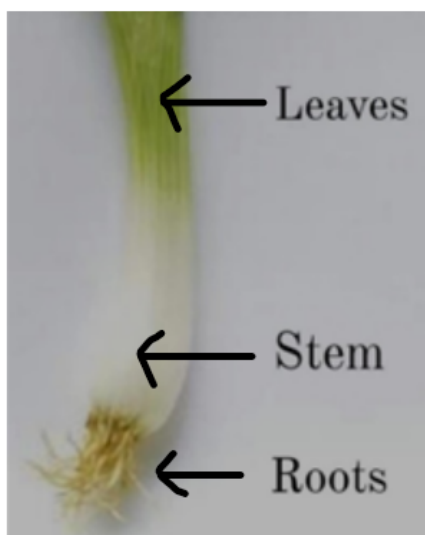


Figure 1. Figure illustrating the structure of *Allium fistulosum*, identifying the roots, stem and leaves.

The purpose of this investigation is to determine if the solutions with different concentrations of ascorbic acid have any effect on the regrowing of *Allium fistulosum* by measuring its growth through the final root lengths and height of the plant. Through the research question: To what extent does the different concentrations of ascorbic acid (0.00 mg/ml, 0.25 mg/ml, 0.50 mg/ml, 0.75 mg/ml, 1.00 mg/ml) affect the growth of *Allium fistulosum* measured through the change in root length (cm) and leaves height (cm) over 14-day period experiment?

2. Materials and method

For this experiment, 15 green onions were used as samples, each numbered from #1-#15. To ensure the reliability of the data and to minimize the amount of random errors, each ascorbic acid concentration was tested on 3 trials. Each *Allium fistulosum* sample was randomly assigned either to one of the 4 different ascorbic acid concentrations or to a control group that did not receive any ascorbic acid. Firstly, every green onion's longest leaf and 3 longest roots were measured using a 30cm ruler for data collection. After that, for each sample it was located where the roots met the meristem and from that point, using a kitchen knife, they were cut at 4 centimeters (measured using a ruler). They were each placed on a labeled plastic cup that was later filled with 60 milliliters of the solutions containing their assigned concentrations. The choice of the volume of the solutions given (60 milliliters) was made based on preliminary experiments to determine the exact amount of water needed to be certain that all the roots and the base of the stem were completely covered by The solution. For each solution 500mg of powdered Vitamin C was dissolved in 500 milliliters of room temperature tap water (26°C) and then diluted to create the concentrations of 0.25mg/ml, 0.50mg/ml, 0.75mg/ml and 1.00mg/ml.

Each of the numbered green onions were then divided into the solution groups,

- #1, #2 and #3 = 0.00mg/ml concentration of ascorbic acid (control group)
- #4, #5 and #6 = 0.25mg/ml concentrate of ascorbic acid
- #7, #8 and #9 = 0.50mg/ml concentration of ascorbic acid
- #10, #11 and #12 = 0.75mg/ml concentration of ascorbic acid
- #13, #14 and #15 = 1.00mg/ml concentration of ascorbic acid

After receiving their solutions, all the samples were placed in the same area to ensure they would all be exposed to the same environmental conditions, including light availability and temperature. All the plants had about 6 hours of sunlight exposure every

day and the environmental temperature had little variation. To prevent contamination of water and to ensure the ascorbic acid concentrations wouldn't be affected throughout the experiment, the solutions were changed everyday and replaced by new ones with the exact same concentration. The experiment lasted for 14 days and at the end they were removed from the solutions, lightly dried in paper towels and measured again for the final data collection.

1.1 Data Collection

Initial data was collected before the green onions were cut and placed on the determined solutions. For each onion, the 3 longest roots and the highest leaf were measured using a ruler. The green onions grew in the solutions for 14 days and at the end of this period, new measurements of the 3 longest roots and highest leaf were taken to determine the growth of the onions. Thus, it was possible to compare how the different solutions affected the growth of the green onions.

2. Hypothesis

Alternative hypothesis (H_1): If the ascorbic acid acts on the differentiation of cells, then when used in a solution, it will increase the growth rate of the green onions because its antioxidant and cell division promoting properties will help the stem cells present in the meristem differentiate and regrow the part of the plant that was removed.

Following those trends, it is expected that the green onions with the highest ascorbic acid concentration will have the biggest growth during the determined period. Consequently, the ones growing with solutions containing the lowest concentrations of ascorbic acid will have the smallest growth after the same period.

Null hypothesis (H_0): The concentrations of ascorbic acid will not affect the growth rate of the green onions.

3. Results and analyses

3.1 Raw Data

Table 1. Table containing the initial measurements of the *Allium fistulosum*. These measurements include the 3 longest roots, the mean root length and the highest leaf from each sample.

Numbered <i>Allium fistulosum</i>	Ascorbic Acid Concentration	Root 1 Length (cm)	Root 2 Length (cm)	Root 3 Length (cm)	Initial Mean Root Length (cm)	Highest Leaf Length (cm)
sample 1	0.00 mg/ml	16	15.5	15	15.5	36.5
sample 2		12.4	12	11	11.8	32.6
sample 3		14.5	10.3	10	12.3	35.5
sample 4	0.25 mg/ml	14	13.6	13	13.5	37
sample 5		20	17.2	15	17.4	36.7
sample 6		14.5	12.5	11.6	12.9	33
sample 7	0.50 mg/ml	21.5	12.5	10.1	15.2	16.5
sample 8		10.7	10.5	10	10.4	36.1
sample 9		17.5	11.4	11.2	13.4	38
sample 10	0.75 mg/ml	15.8	13.2	7.1	12	15
sample 11		20	16.5	16.3	17.6	15.8
sample 12		21	19.7	17.5	19.4	14.9
sample 13	1.00 mg/ml	17	15.9	13	15.3	14.5
sample 14		12.6	10.8	10.3	11.2	17.4
sample 15		15.2	14.5	13.4	14.4	40.5

Table 2. Table containing the final measurements of the *Allium fistulosum* on day 14 of the experiment. These measurements include the 3 longest roots, the mean root length and the highest leaf from each sample. It also includes the ascorbic acid concentration that the samples were exposed to.

Numbered Allium fistulosum	Ascorbic Acid Concentration	Root 1 Length (cm)	Root 2 Length (cm)	Root 3 Length (cm)	Final Mean Root Length (cm)	Highest Leaf Length (cm)
sample 1	0.00 mg/ml	16.1	15.7	15.3	15.7	5.3
sample 2		13.7	12.6	11.3	12.5	5.9
sample 3		14.9	11.4	11.1	12.5	4.9
sample 4	0.25 mg/ml	16.5	15.1	13.7	15.1	6.8
sample 5		21.2	18.3	16	18.5	6.4
sample 6		16.8	14.2	13.5	14.8	6.9
sample 7	0.50 mg/ml	24.5	16.3	12.2	17.7	7.2
sample 8		14.9	13.1	13.2	13.7	8.1
sample 9		24	13.2	11.3	16.2	7.8
sample 10	0.75 mg/ml	18.4	16.2	13.5	16	8.4
sample 11		24.9	23.9	23.6	24.2	9.7
sample 12		30	28.5	18.1	25.5	9
sample 13	1.00 mg/ml	23.8	22.4	21.1	22.4	10.9
sample 14		18.1	17.4	17.2	17.6	9.5
sample 15		19.5	18.9	18.8	19.1	8.1

Table 3. Table showing the comparison between the initial (day 0 of the experiment) and final (day 14 of the experiment) mean root length of each sample, using the average measurements from the 3 longest roots.

Numbered green onions	Ascorbic Acid Concentration	Initial Mean Root Length (cm)	Final Mean Root Length (cm)	Change in Mean Root Length (cm)
1	0.00 mg/ml	15.5	15.7	0.2
2		11.8	12.5	0.7
3		12.3	12.5	0.2
4	0.25 mg/ml	13.5	15.1	1.6
5		17.4	18.5	1.1
6		12.9	14.8	1.9
7	0.50 mg/ml	15.2	17.7	2.5
8		10.4	13.7	3.3
9		13.4	16.2	2.8
10	0.75 mg/ml	12	16	4
11		17.6	24.2	6.6
12		19.4	25.5	6.1
13	1.00 mg/ml	15.3	22.4	7.1
14		11.2	17.6	6.4
15		14.4	19.1	4.7

3.2 Data Analysis

To better analyze how the different concentrations of ascorbic acid affected the growth of the *Allium fistulosum*, the average root length and the highest leaf for the 3 trials will be determined to provide a better analysis of the effect of the different concentrations. Furthermore, the standard deviation of these values will be calculated in order to determine the dispersion of this data.

The average root length of the different concentrations was calculated using the formula:

$$\frac{\text{Sum of the mean root lengths from all the trials in each concentration group}}{\text{number of trials in each concentration group}}$$

The standard deviation of each concentration was calculated using the formula:

$$S = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n - 1}}$$

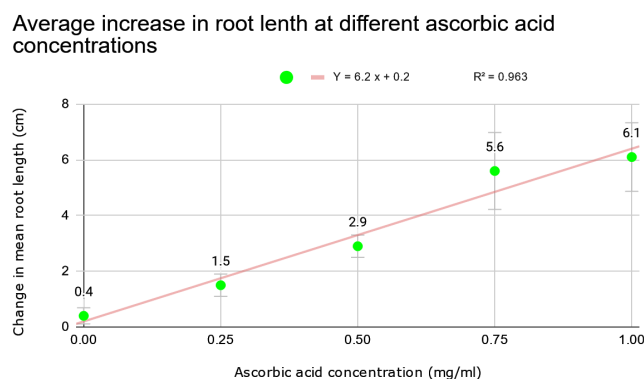
3.3 Root Growth Analysis

Table 4. Table displaying the change in mean root length from the beginning of the experiment (initial condition) to the end of it (final condition) for each of the concentrations group. The average root length for each concentration was calculated using the mean values from each trial of the concentration groups.

Ascorbic Acid Concentration (mg/ml)	Concentration Initial Mean Root Length (cm)	Concentration Final Mean Root Length (cm)	Change In Concentration Mean Root Length (cm)
0	13.2	13.6	0.4
0.25	14.6	16.1	1.5
0.5	13	15.9	2.9
0.75	16.3	21.9	5.6
1	13.6	17.7	6.1

The change in mean root length represents the amount of growth, measured in centimeters, that the roots from each sample had. So, the groups that had the biggest change in mean root length are the ones that had the greatest growth. While interpreting this table, it is possible to observe a pattern, where the change in mean root length is increasing as the ascorbic acid concentration increases. Based on this data, a graph was plotted to better visualize and analyze this relationship.

Graph 1. Graph showing the average increase in root length (difference between the initial and final root lengths) at the different concentrations, that is represented by the green points. The graph also contains error bars, calculated using the standard deviation from each concentration, and a trendline represented by the light red line.



The change in mean root length is the difference between the initial mean root length (measured before the experiment) and the final mean root length (measured at the end of the experiment). Thus, this graph is a visual representation of how much the roots had grown in each of the concentration groups. The trend seen in tables 3 and 4 and in this graph shows that the root length growth increases linearly with increasing the concentration of ascorbic acid. Analyzing the data, it is possible to observe that as the concentration of vitamin C increases, the change in mean root length also increases. For example, the green onions that grew in the solution that had the highest concentration of ascorbic acid (1 mg/ml) were the ones that had the biggest growth in mean root length, with a mean growth of 6.1 cm. In contrast, in the control group,

where ascorbic acid was not added, the mean growth was only 0.4 cm. This is a consistent trend throughout the graph, in which the concentration of ascorbic acid is directly correlated to the growth of the roots.

The graph also contains a trendline which allowed me to calculate its coefficient of determination (R^2). The R^2 determines the proportion of variation in the dependent variable that can be attributed to the independent variable. In this case, the R^2 value equals 0.963 which suggests very strong correlations between the variables according to the commonly accepted range of $0.75 < R^2 < 1$. Therefore, we can conclude that there is a positive and strong correlation between the change in mean root length and the amount of ascorbic acid added to the solution.

To test the statistical significance of this relationship

the one-way analysis of variance (ANOVA) test was performed. The ANOVA test is used to determine if there is any difference between the means of different groups, thus it can be used to show if there are statistically differences between the data collected from the different ascorbic acid concentration groups.

The following hypothesis were used for the ANOVA test:

Null Hypothesis (H_0): There is no statistically significant difference between the mean root growth length of *Allium fistulosum* when given a different ascorbic acid concentration solution.

Alternative Hypothesis (H_1): There is a statistically significant difference between the mean root growth length of *Allium fistulosum* when given a different ascorbic acid concentration solution.

Table 5. Table containing the data used in the ANOVA Test.

ANOVA Test Summary			
Groups	Count	Mean (μ)	Standard Deviation (σ)
0 mg/ml	3	0.4	0.2357
0.25 mg/ml	3	1.5	0.3299
0.50 ml/ml	3	2.9	0.3299
0.75 mg/ml	3	5.6	1.1264
1.00 mg/ml	3	6.1	1.0077

Table 6. Table containing the results of the ANOVA Test, illustrating the variations between groups, within groups, and total. SS = sum of squares due to the source is, df = degree of freedom, MS = mean sum of squares due to the source, F = ratio of between group variation and within group variation.

ANOVA Test Results					
Source of Variation	SS	df	MS	F	P-value
Between Groups	46.02	4	11.505	22.493	0.00003
Within Groups	5.115	10	0.511		
Total	51.135	14			

Considering that the P-value obtained after performing the one-way ANOVA Test is 0.00003 and

the significance level to be 0.05, the null hypothesis will be rejected as ($P < 0.05$). Thus, the alternative

hypothesis is accepted, suggesting that there is a statistically significant relationship between the growth of the roots and the Vitamin C concentration in the solutions.

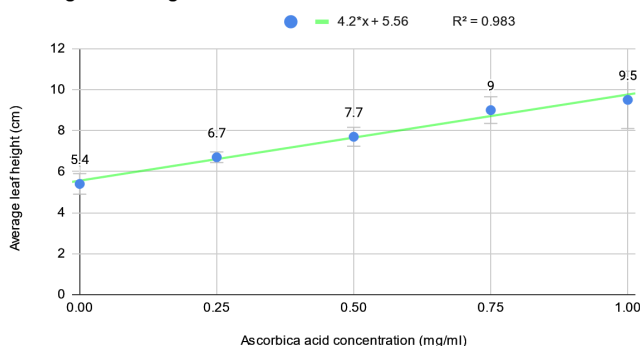
3.4 Leaf Growth Analysis

Table 7. Table showing the leaf growth at different concentrations of ascorbic acid by measuring the average height of the tallest leaf from the 3 trials in each concentration. The standard deviation of this data was also determined.

Ascorbic Acid Concentration (mg/ml)	Highest Leaf Average (cm)	Standard Deviation
0	5.4	0.5
0.25	6.7	0.26
0.5	7.7	0.46
0.75	9	0.65
1	9.5	1.4

Graph 2. Graph showing the average leaf height at the different concentrations of ascorbic acid, represented by the blue points. The graph also contains error bars, calculated using the standard deviation from each concentration, and a trendline represented by the green line

average leaf height at different ascorbic acid concentrations



This graph was plotted with the measurements from the highest leaf of each sample and calculating the average height for the concentrations. Analyzing this data, it is possible to observe a correlation between the 2 variables. The trend seen in this graph shows that as the concentration of vitamin c increases, the higher the leaves grow. The trendline in the graph allows us to analyze the coefficient of determination (R^2), as it was previously used to analyze the average root length. Given that R^2 equals 0.985 and observing that this value is very close to 1 and that $0.75 < R^2 < 1$, then we can conclude that there is a strong correlation between the variables. Thus, this data shows a positive and strong correlation between the height of the leaves and the amount of ascorbic acid added to the solution.

4. Evaluation

Table 8. Table expressing the limitations of the experiment by evaluating the error committed, type of error, analyzing the significance of error and presenting a method of improvement.

Error	Type of error	Significance	Method of Improvement
Measurement of the <i>Allium fistulosum</i>	Systematic error	The measurement of the leaves and roots were done using a ruler, which is a limited and not a very accurate method of determining these measurements as it relies on eye sight and is subjected to human error.	More precise length measuring tools could have been used such as a digital micrometer or caliper.
Size differences between <i>Allium fistulosum</i>	Systematic error	Plants need meristematic cells to grow due to their great capacity of multiplication and differentiation. All samples were cut at the same height (4 cm from root base), but the slight difference from the thickness in each sample affects the number of meristematic cells available for differentiation, consequently, affecting the growth rate of the plants.	The mass of each sample could have been measured to better compare the size of each plant and reduce the disparity between them.

Control of external variables	Random error	The growth of <i>Allium fistulosum</i> is affected by the light availability and environmental temperature. All the samples were placed at the same area and exposed to the same uncontrolled conditions.	The experiment could have been conducted in a controlled environment with controlled temperature. In order to prevent light availability disparity, lamps could have been used as a light source.
Limited data	Limitation	The samples were only measured at the beginning of the experiment and at the end of the fourteen days, which only gave me the initial and final conditions of the plants.	The samples could have been measured more often throughout the experiment to show the growth rate of the plants.
Duration of the experiment	Limitation	The experiment had the duration of 14 days, while the <i>Allium fistulosum</i> could still grow in an aquatic environment.	The experiment could be extended if the samples were transferred to a pot filled with good quality potting soil. This way it would be possible to analyse the long-term effect of the ascorbic acid on their growth.
Frame of observation	Limitation	This experiment focused only on analysing the roots and leaves growth, but as mentioned on the Background Information vitamin C is involved in other processes within the plants that could also be affected with the increase of its concentration.	Other aspects of the plants could be analysed, such as the chlorophyll content or photosynthesis rate, to further understand the impact of the ascorbic acid.

5. Conclusion

The aim of this experiment was to determine if the solutions with different concentrations of ascorbic acid had any effect on the regrowing of *Allium fistulosum*, with my hypothesis being that the samples in the solutions containing the highest concentrations of ascorbic acid would have the highest growth and the ones with the lowest concentrations would have the lowest growth. After analyzing the experiment's results and statistical tests, I can conclude that the ascorbic acid concentrations did affect the growth of the *Allium fistulosum*. Therefore, the null hypothesis is rejected and, consequently, the alternative hypothesis is accepted. This effect is shown through all the tables and graphs in this investigation.

Firstly, there was a significant root length increase shown in graph 1, with both the one-way ANOVA test, in which $P = 0.00003 < 0.05$, and the R^2 tests, in which $R^2 = 0.963 > 0.075$, proving that the average root length increase and the ascorbic acid concentrations had a positive strong correlation. Then, in graph 2 the R^2 test determined that there was an incredibly strong correlation between the average leaf height and the different ascorbic acid concentrations, with R^2 equaling 0.983.

One of the explanations for the outcome of this experiment is that the meristematic tissue in *Allium fistulosum* was preserved after the cut, so the part of the plant structure that carries the most amount of stem cells was not damaged. Seeing that the stem cells have a great differentiation and multiplication capacity and that the ascorbic acid also takes part in the process of cell division and differentiation, then the solutions containing the ascorbic acid could have helped to potentialize even more the plant's capacity of differentiation and cell division, as those processes are extremely important in the growth of new tissue (PACIOLLA et al, 2019).

Majeed (2019) in a similar study has shown that ascorbic acid had great impact in the growth and development of other vegetables and have reached identical results to my own experiment, which further contributes to the possibility of increasing the use of ascorbic acid for the growth of plants in the future. However, *Allium fistulosum* has its own individual species characteristics, so additional research is needed to better understand how ascorbic acid might behave on other species or how a different range of concentrations might affect the development of the plants.

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