## Examiner's commentary

This is an interesting and well-written essay on a topic with obvious biological significance. The method is described in detail and key elements are justified by reference to published work. The research question is clear and precise, and this helps to frame the essay and maintain its focus. The background is relevant and precise but could be more expansive making more use of material from an extensive bibliography. Data analysis is conducted competently, and the approach used is well explained and justified. Graph 3.3 is perhaps the most effective and direct illustration, relating the findings to the research question. Other graphs are perhaps not as effective and deviate somewhat from the main message of the essay. Table 3.3 gives a precise and valuable overview of data processing and the outcomes of the statistical analysis, and the line of argument linking the conclusions with the statistical significance of the data is well supported. The discussion is both detailed and well supported by references to the sources. There is a sound critical evaluation where important limitations are identified and discussed in the light of published findings although the subdivision of this section into "experimental aspects" and "real life applications" is a bit misleading. Overall this is a coherent study backed up by strong data collection and relevant literature.

# The study on the effects of the concentration of 6-Benzylaminopurine on the leaf growth of *Canavalia gladiata*

**Research Question:** : How does the concentration of 6-Benzylaminopurine applied exogenously influence the leaf growth of *Canavalia gladiata* grown in soil as measured by the growths in number of leaves, length and width of the longest leaves?

**Subject:** Biology

**Word Count:** 3957

# Table of Contents

# 1. Introduction

## I. Cytokinins

Cytokinin is a type of plant hormone that induces cell division. D-cyclins are a group of proteins that trigger the shift in cell cycle phases from G1 to mitotic phase. Cytokinins are responsible for promoting transcription of *CycD3* that translates D-type cyclins, inducing cell division, enlargement of apical meristem and shoot growth (D'Agostino and Kieber 359). Cytokinins are also responsible for apical dominance with auxin, another plant growth hormone. The ratio of cytokinins and auxin determines if axillary bud growth at the junction of a leaf and stem will be induced or inhibited. If the concentration of cytokinins is higher than that of auxin, the axillary bud growth will be induced (Allot 422).

### I.I Cytokinins as fertilizer

Cytokinins are being used as a source of fertilizer to increase crop yields in agriculture. Transgenic cotton plants had suppressed cytokinin dehydrogenase, an enzyme that decreases the level of cytokinins. The transgenic cotton with the highest suppression of cytokinin dehydrogenase resulted in the greatest yield, more branches and bigger seeds (Jameson et. al). This case study revealed the positive influences of cytokinins so that farmers can utilize to increase their crop yields.

### I.II 6-Benzylaminopurine

6-Benzylaminopurine is a naturally produced adenine-type plant hormone (abbreviated as BAP). BAP is an organic molecule that has functional groups, benzene and amino, bonded to a purine that is one of the widely studied cytokinins. The chemical structure of a BAP is shown in Figure 1.1.

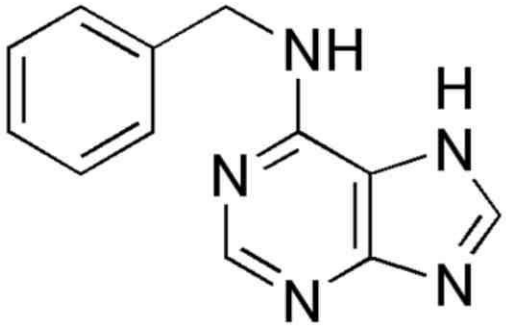



Figure 1.1 6-Benzylaminopurine[1] (PubChem)

[1] Unless otherwise indicated by a citation, all graphs, charts, and tables were created by me

## II.*Canavalia gladiata*

*Canavalia gladiata* is also known as sword beans involved in legume family. *C.gladiata* grows in tropical areas and can tolerate 12°C to 36°C. South Korea, where temperature varies from -10°C to 40°C by seasons, harvests *C.gladiata* in greenhouses to maintain a tropical climate (World). *C.gladiata* beans are used as health functional food and leaves as tea to treat daily illnesses like digestive problems and rhinitis (Gan, Ren-You et. al).

## III. Research Question

Not only farmers, but also people who raise plants at home would be benefited by using cytokinins. However, the effects of exogenous application of cytokinins on plants grown in soil are rarely or not studied by scientists, whereas *in vitro* propagation of plants using cytokinins and algal medium is a popular investigation. Therefore, in order to increase practicality of using cytokinins to promote plant growth so that they can be used in real life, different concentrations of 6-benzylaminopurine and their effects on the leaf growth of *Canavalia gladiata* grown in soil is examined.

: How does the concentration of 6-Benzylaminopurine applied exogenously influence the leaf growth of *Canavalia gladiata* grown in soil as measured by the growths in number of leaves, length and width of the longest leaves?

## IV. Approach

Five different concentrations BAP (0, 2, 4, 6, 8 ppm) are tested to observe a correlation between BAP concentrations and the leaf growth of *C.gladiata*. The number of leaves, lengths and widths of longest leaves of *C.gladiata* are measured as dependent variables. Number of beans yielded from plants are not measured due to limited time. The results are compared with that of similar investigations such as, *in vitro* multiplication of shoots of different plant species in medium containing different BAP concentration, because an investigation looking at the effects of adding different BAP concentrations on *C.gladiata* grown in soil is not done by any researcher.

## V. Hypotheses

**A.** The leaf growth of *C.gladiata* will positively correlate with the concentrations of BAP because BAP promotes growth of plants by inducing cell division so more numbers of leaves and larger leaves would be formed with increasing BAP concentration. In addition, the exogenous addition of cytokinins resulted in a better growth by having a large apical meristem and more numbers of shoots, indicated earlier in I. Cytokinins.

***$H_o$* Null hypothesis:** There is no significant correlation between the BAP concentration and the average final number of leaves, the average final width and length of the longest leaves.

***$H_1$* Alternative hypothesis:** There is a significant correlation between the BAP concentration and the average final number of leaves, the average final width and length of the longest leaves.

## VI. Safety and Ethics

The range of BAP concentration does not cause plant necrosis, as the range was also used for *in vitro* propagation of other plant species. To humans, BAP causes irritation in case of eye, skin contacts and inhalation and could be hazardous when ingested. Hazardous when ingested does not mean that *C.gladiata* treated with BAP is not edible because BAP is metabolized as a hormone. Although dilute BAP is used, lab coats, specs and possibly facial masks should be worn. BAP should be disposed into a separate waste container, as it is hazardous to aquatic organisms (PubChem). Only 4mL sodium hydroxide is added indirectly to plants, so this would not negatively influence the growth of *C.gladiata*. Sodium hydroxide is corrosive in case of eye, skin contacts, inhalation and ingestion so gloves should be worn, too. Sodium hydroxide should be disposed into a separate waste container (LabChem).

# 2. Methodology

## Part I. Variables

**1. Independent variable:**

The concentration of BAP solution is an independent variable: 0, 2, 4, 6 and 8 ppm[2]. This range is particularly chosen as many investigations looking at the effects of BAP concentration on *in vitro* propagation plants used this range (Arab et. al 82; Arinaitwe).

**2. Dependent variable:**

The number of leaves, lengths and widths of the longest leaf are measured using a 30cm ruler as dependent variables to observe the leaf growth of *C.gladiata*. The leaf growth is measured instead of the shoot growth because *C.gladiata* is a vine so the length of stem is difficult to measure. The length and width of the longest leaf are measured for ease of tracking the growth of leaves.

**3. Controlled variables:**

1) Watering: Water *C.gladiata* 75mL every two days until two shoots are formed then water them 75mL every three days. Water directly influences the growth of *C.gladiata*. Lack of water could cause wilting (Petruzzello) and excessive water could cause rotting roots, which could kill the plants (Botts).

2) Volume of BAP solution: 40ml of a corresponding BAP solution is added once a week. The volume of BAP solution should be the same, as the concentration is different. Since BAP solution is mostly distilled water, adding additional or less volume of BAP solution would not control the amount of water added to plants.

3) Amount of sunlight and temperature of the environment: Place the pots in the veranda besides the windows where enough sunlight and warmth are provided. Since the experiment proceeds in the late June to the early August, temperature of the veranda is within the tolerable temperature range of *C.gladiata*: 12 to 36°C (Ken Fern). Sunlight provides light energy for photosynthesis, so plants with more sunlight would grow better. Therefore, the amount of sunlight should be controlled to obtain a clear correlation between two set variables.

[2] ppm = parts per million = milligrams per liter

4) Type of soil: Soil is a growth medium so it should provide the same fertility and nutrients to attain a clear correlation between the BAP concentration and the leaf growth of *C.gladiata*. The same type of soil that does not contain fertilizers bought from the same nursery store is used for all *C.gladiata* plants.

## Part II. Experminet

| Material or Equipment | Quantity | Uncertainty (if applicable) |
|---|---|---|
| *C.gladiata* beans | 40 | - |
| Culture soil without fertilizers | 10 kg | - |
| 99.0% pure 6-Benzylaminopurine powder | 1 g | - |
| 1M sodium hydroxide solution | 10 mL | - |
| Distilled water | 6 L | - |
| Pots | 5 | - |
| 1L Beaker | 1 | ±2.5mL |
| 5ml Pipette | 1 | ±0.5mL |
| Glass stirring rod | 1 | - |
| Analytical balance | 1 | ±0.00005g |

**Table 2.1 – Materials and Equipment**

**Preparation**

1. Measure 80mg of BAP using an analytical balance.
2. Put 80mg BAP into 1L distilled water in a 1L beaker.
3. Measure 4mL 1M sodium hydroxide solution using a 5mL pipette and put it into the 1L beaker.
4. Stir the solution with a glass stirring rod until all BAP powder is dissolved.
5. Using 80 ppm BAP solution, a specific concentration of BAP is obtained by dilutions.

| Concentration of BAP (ppm) | Volume of 8 ppm BAP solution (mL) | Volume of distilled water (mL) |
|---|---|---|
| 2 | 250 | 750 |
| 4 | 500 | 500 |
| 6 | 750 | 250 |
| 8 | Diluting 100mL of 80 ppm BAP solution with 900mL distilled water. | |

**Table 2.2 Volume required to make a specific concentration of BAP solution**

**Growing**

1. 25 *C.gladiata* beans are completely soaked in 20°C tap water for 24 hours.
2. *C.gladiata* beans' testas are slightly cut with a scalpel to encourage their sprouting.
3. Replace each of five beans into a pot with culture soils 15cm deep where their microphyles are in direct contact with soil.
4. Pots are placed at the outdoor veranda beside the windows where there is enough sunlight and is warm enough for tropical plants to grow.
5. 75mL tap water is added every two days until two leaves are formed from a *C.gladiata* bean.
6. Once two leaves are formed, the initial lengths and widths of the longest leaves are measured with a 30cm ruler. Initial measurements of these dependent variables are recorded in Day 0.
7. 40mL of corresponding concentrations of BAP are added once a week for three weeks and 75mL of tap water is added every three days throughout the experiment.
8. Number of leaves, lengths and widths of the longest leaf are measured on every Tuesday, Friday and Sunday throughout the experiment.
9. Repeat Step 1 - 8 but with 15 beans in Step 1 and 3 beans in Step 3; there are eight trials in total for this experiment.

## Part III. Data Treatment

The averages of trials and standard deviations of the final number of leaves, final length and width of the longest leaves are calculated. The Pearson Product Moment Correlation Coefficient test (PPMCC) is used to determine if the correlation between BAP concentrations and the leaf growth (average final number of leaves, average final length and width of the longest leaves) is strong. Then t-test is conducted to test for the significances of these correlations.

1.Calculate PPMCC value (= r) using the formula below.

$$r = \frac{S_{xy}}{S_x S_y}$$

$$S_{xy} = \sum xy - \frac{(\sum x)(\sum y)}{n}$$

$$S_x = \sqrt{\sum x^2 - \frac{(\sum x)^2}{n}}$$

$$S_y = \sqrt{\sum y^2 - \frac{(\sum y)^2}{n}}$$

n = pairs of data = 5

x = Concentration of BAP in ppm

y = Averages of final number of leaves or lengths or widths of the longest leaves

2. Calculate $t^*$ using the formula below.

$$t^* = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

$$r = PPMCC\ value\ \ n = Pairs\ of\ data\ = 5$$

3. Calculate tCdf, Student's T-distribution, using Ti-nspire calculator, setting the lower bound as -9.E999 and the upper bound as $t^*$ from Step 2 and the degree of freedom (5 - 2 = 3) as 3.

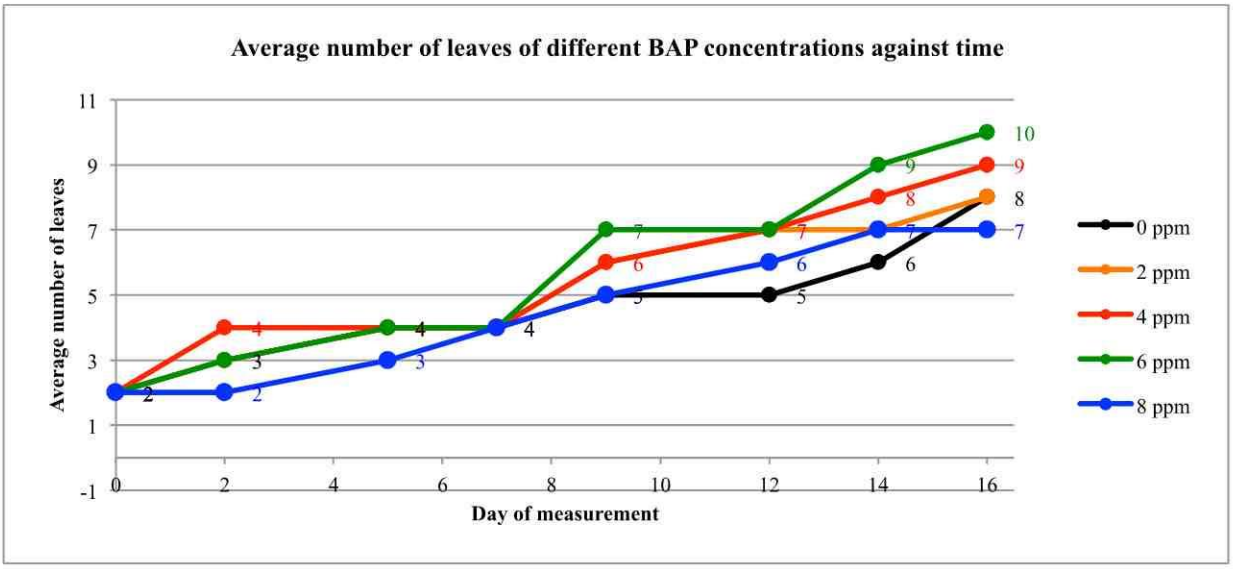
4. Subtract the value calculated in Step 3 from 1 and multiply that by 2.

5. If the value calculated in Step 4 is smaller than the significance level, 0.05, reject $H_o$.

# 3. Data Analysis

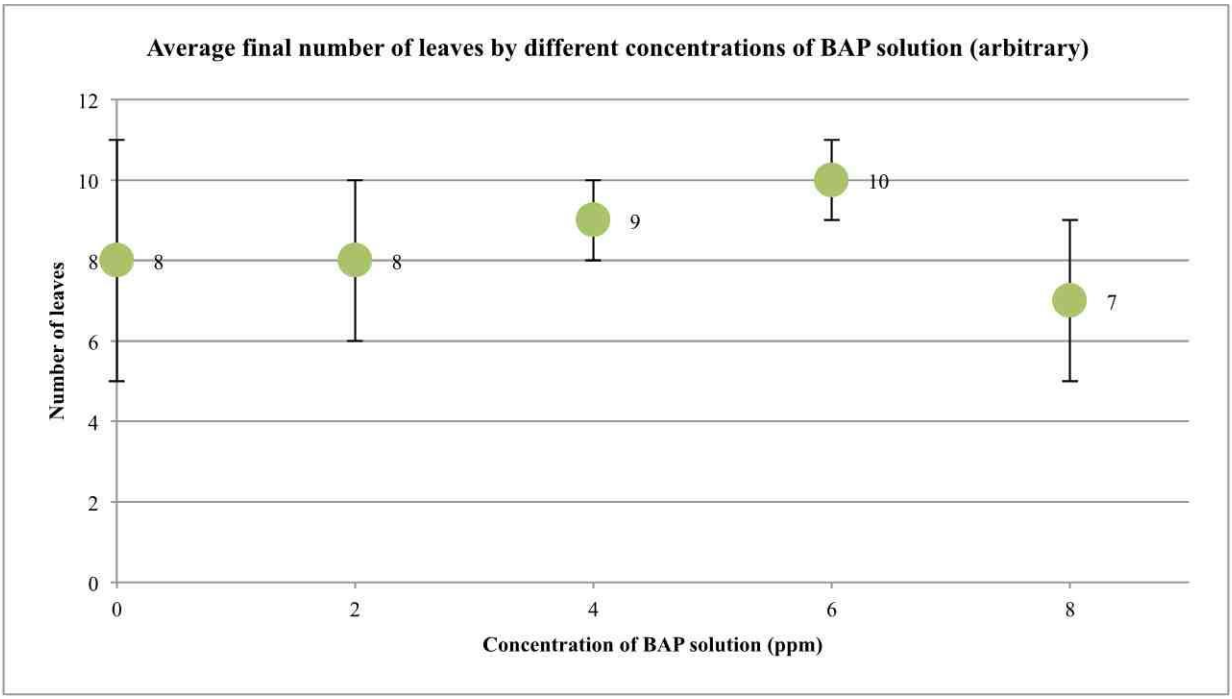
| Day | Number of leaves | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Trial 1** | **Trial 2** | **Trial 3** | **Trial 4** | **Trial 5** | **Trial 6** | **Trial 7** | **Trial 8** |
| 0 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 2 | 5 | 5 | 2 | 2 | 2 | 2 | 2 | 2 |
| 5 | 6 | 5 | 3 | 2 | 2 | 5 | 2 | 5 |
| 7 | 6 | 5 | 4 | 2 | 2 | 5 | 5 | 5 |
| 9 | 7 | 5 | 5 | 3 | 2 | 5 | 5 | 5 |
| 12 | 7 | 5 | 5 | 3 | 2 | 8 | 8 | 5 |
| 14 | 7 | 9 | 5 | 3 | 2 | 9 | 8 | 8 |
| 16 | 10 | 9 | 8 | 6 | 2 | 9 | 10 | 10 |

**Table 3.1 Sample of raw data – Number of leaves treated without BAP solution against time; rest attached in the Appendices**




**Graph 3.1 - Average number of leaves of different BAP concentrations against time**

The initial number of leaves at all concentrations was 2. On Day 2, *C.gladiata* plants treated with 4 ppm BAP solution had the most leaves, which was 4, whereas plants treated with 8 ppm BAP solution had the least leaves, which was 2. Additionally, plants treated with 8 ppm BAP solution have the least number of leaves upto Day 7. On the other hand, plants treated with 6 ppm BAP solution have the greatest number of leaves since Day 5. The growth in number of leaves is most gradual for the control, which can be seen from a gradient of the black line graph.




**Graph 3.2 - Average final number of leaves by different concentrations of BAP solution**

The average final number of leaves are rounded upto nearest whole numbers because number of leaves can only be a whole number. In general, the number of leaves increases with an increasing BAP concentration, whereas there is a drop from 6 ppm to 8 ppm. *C.gladiata* plants treated with 6 ppm BAP solution had the most average final number of leaves (10) and plants treated with 8 ppm BAP solution had the least average final number of leaves (7). The final numbers of leaves are not different for control and plants treated with 2 ppm BAP solution (8).

The black error bars in Graph 3.2 indicate standard deviations. Standard deviations are significant for the control, 2 ppm and 8 ppm, decreasing the reliability of the averaged value. The unequal distribution of water and essential nutrients to plants placed in one pot could have led to great standard deviations, as plants that received more water and nutrients from the soil would have produced more leaves.

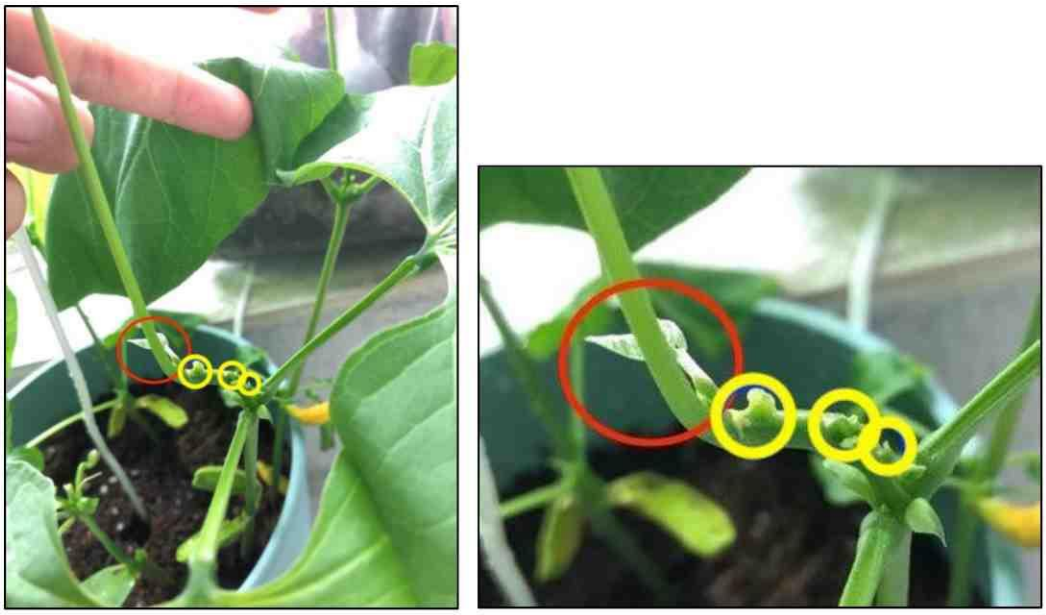
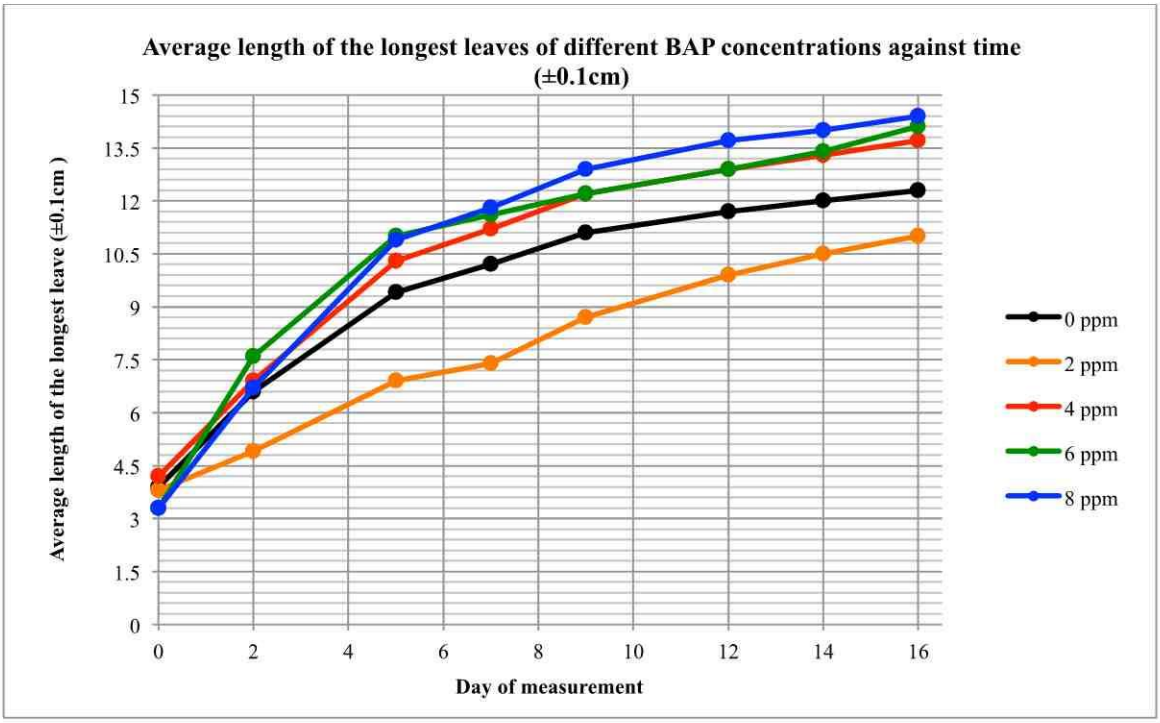


**Figure 3.1 – Qualitative observation 1**

One *C.gladiata* plant treated with 2 ppm BAP solution did not produce as many leaves as other plants grew in the same pot possible due to a lateral bud formation. Qualitative observation shown in Figure 3.1 displays that about four lateral buds, indicated as circles, are growing whereas only one of them, indicated as a red circle, seems to be growing into a mature leave.

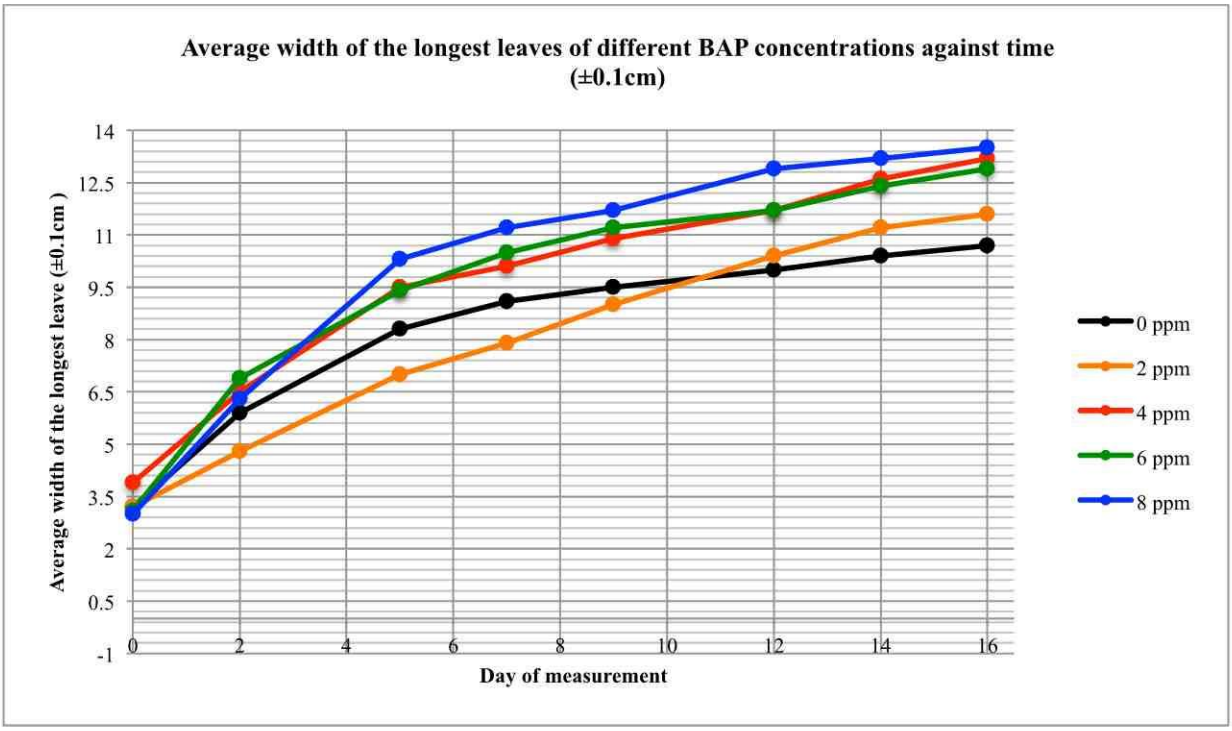
**Figure 3.2 – Qualitative observation 2**

This is a picture of one *C.gladiata* plant treated with 8 ppm BAP solution. About seven shoots are growing from one apical meristem. One to two leaves are growing from each shoot but at the end of the experiment, none of them actually grew into a mature leaf.



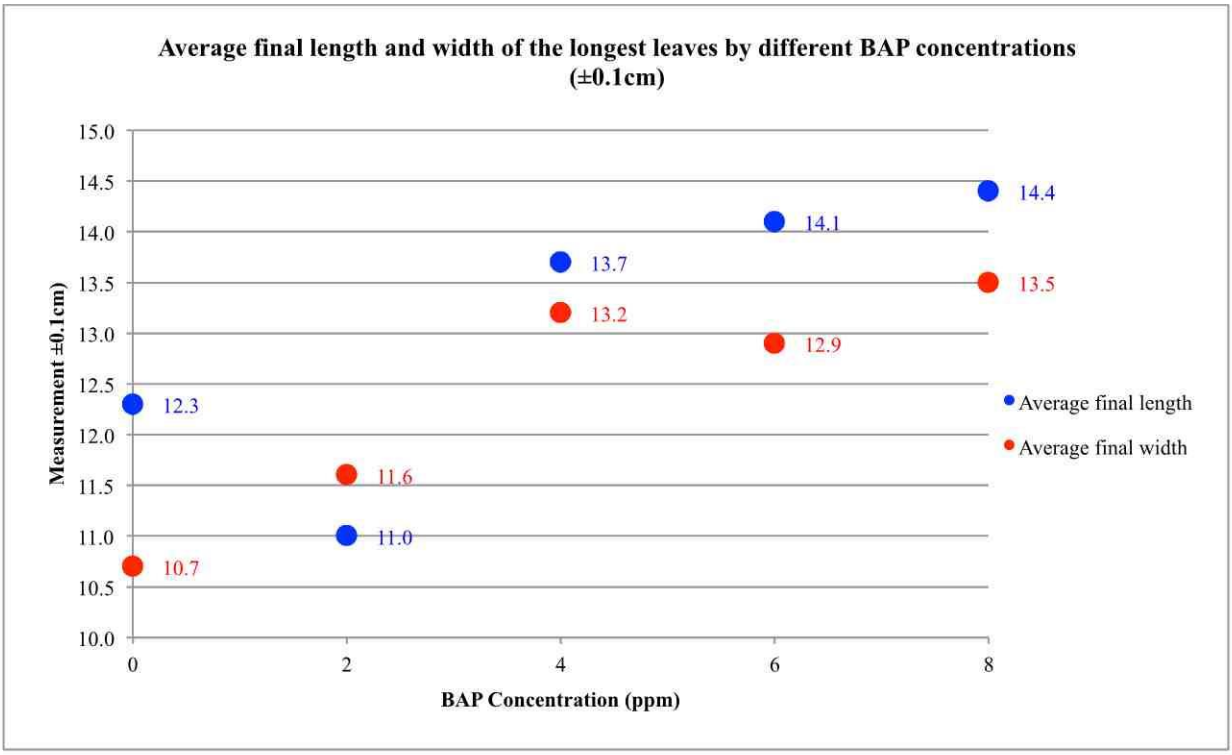

**Graph 3.3 - Average length of the longest leaf of different BAP concentrations against time (±0.1cm)**

The growth is most fast for plants treated with 6 ppm BAP solution upto Day 5 while it is most gradual for plants treated with 2 ppm BAP solution for the most days. After Day 5, the growth in length of a leaf slows down for all concentrations. The average length of the longest leaf treated with 2 ppm is always smaller than that of other concentrations except the initial measurement. The average length of the longest leaf on Day 16 is the greatest for 8 ppm, which is 14.4cm.




**Graph 3.4 - Average width of the longest leaves of different BAP concentrations against time (±0.1cm)**

The growth in average width of the longest leaf has a similar trend with the growth in average length. The fastest growth is observed in plants treated with 8 ppm for the most days. The growth of width of the longest leaf for all concentrations slows down since Day 5 like it did in Graph 3.3. The average width of the longest leaf in Day 16 is the greatest for 8 ppm (13.5cm) and the smallest for the control (10.7cm).




**Graph 3.5 – Average final length and width of the longest leaves by different BAP concentrations (±0.1cm)**

In general, both the average final length and width of the longest leaves increase with an increasing concentration of BAP solution. Plants treated with 2 ppm BAP has a smaller average final length than that of the control by 1.3cm, deviating from the general trend. However, the average final width is greater for 2 ppm than that of the control by 0.9cm. Thus, *C.gladiata* plants treated with 2 ppm could have grown horizontally rather than vertically. The average final width of the longest leaf for 6 ppm is shorter than that of 4 ppm by 0.3cm, deviating from the general trend while the average final length is greater for 6 ppm than that of 4 ppm by 0.3cm. Leaves of *C.gladiata* plants treated with 4 ppm BAP solution could have grown vertically than horizontally. The deviations could be due to morphological adaptations of leaves to maximize photosynthesis in a given environment (Tsukaya).

| Concentration (ppm) | Average final length (±0.1cm) | Average final width (±0.1cm) |
|---|---|---|
| 0 | 2.0 | 0.8 |
| 2 | 1.8 | 2.2 |
| 4 | 1.5 | 1.4 |
| 6 | 0.9 | 1.1 |
| 8 | 0.8 | 1.1 |

**Table 3.2 – Standard deviations of the average final length and width of the longest leaves dy different BAP concentrations (±0.1cm)**

The standard deviations of the average final lengths and widths of the longest leaves are significantly great for the control (0 ppm), 2 ppm and 4 ppm. It is hard to explain why standard deviations are great for particular BAP concentrations. As mentioned previously in Data Analysis of Graph 3.5, standard deviations are likely to be due to biological variabilities.

| | $r$ | $t^*$ | Significance level | p-value | Interpretation |
|---|---|---|---|---|---|
| Average final number of leaves | 0.00 | 0.00 | 0.05 | 0.000 | $H_o$ accepted |
| Average final length of the longest leaf | 0.811 | 2.40 | | 0.0957 | $H_o$ accepted |
| Average final width of the longest leaf | 0.920 | 4.06 | | 0.0270 | $H_o$ rejected |

**Table 3.3 - PPMCC and $t^*$ tests results of each dependent variable (rounded up to three significant figures)**

The *r*-value of average final number of leaves is 0 so it means in statistics that there is no linear correlation between BAP concentration and the average final number of leaves. Since PPMCC tests for linear relationship, having zero for *r*-value could mean that the correlation is not linear though there is a correlation. This makes sense in this investigation because the average final number of leaves increased from 0 ppm (8) to 6 ppm (10) then decreased from 6 ppm (10) to 8 ppm (7).

The *r*-value of average final length of the longest leaves is 0.811, suggesting positive correlation, but p-value is 0.0957, which is greater than the significance level therefore there is not enough evidence to reject $H_o$. However, this does not mean that there is no correlation between BAP concentrations and the average final length of the longest leaf, but it shows that there is no significant evidence of the correlation, suggesting the need of more trials. On the other hand, the *r*-value of average final width of longest leaves is 0.920 and its p-value is 0.0270, which is smaller than the significance level therefore providing enough evidence to reject $H_o$ at the 5% level.

## 4. Discussion

According to Graph 3.2, the average final number of leaves increased with an increasing concentration upto 6 ppm then decreased from 6 ppm to 8 ppm. The results showed that the optimum concentration of BAP solution that would result in the most number of leaves is 6 ppm; not the highest concentration 8 ppm as predicted in hypothesis A. In fact, the optimum concentration of BAP for shoot multiplication of banana plants cultured *in vitro* was 6 ppm. Additionally, the number of shoots generated decreased when the BAP concentration increased from 6 ppm to 8 ppm (Arinaitwe), agreeing with the results of this experiment.

Decreased multiplication of leaves can be explained by hormone homeostasis. Increased BAP or cytokinins level could have induced the activity of cytokinin oxidase, an enzyme that inactivates free cytokinins by hydrolyzing $N^6$-side-chains of adenine type cytokinins such as, BAP (Druege). The cytokinin oxidase activities of transgenic tobacco with increased cytokinin levels was compared with wild type tobacco. It was found that cytokinin oxidase activity was higher for transgenic tobacco than that of wild type (Motyka 1037). Therefore, increased BAP concentrations in *C.gladiata* plants could have resulted an increase in cytokinin oxidase activity, resulting in less formation of leaves.

Both the average final length and width of the longest leaf tend to increase with an increasing concentration of BAP, except several deviations as explained previously in the Data Analysis. The results can be compared with the finding of another similar investigation looked at the effects of BAP concentrations on changes in leaf size of *Epipremnum aureum* cuttings (Benedetto et. al 179). The intermediate concentration, 5 ppm BAP solution, led to the greatest increase in the surface area and biomass of leaves, which do not agree with the results of this experiment, where the highest BAP concentration resulted in the greatest leaf expansion. Moreover, the investigation looked at the effects of BAP concentration on *in vitro* shoot regeneration of *Chlorophytum borivilianum* observed the decrease in shoot lengths at high concentrations of BAP. The investigators explained that this is due to a greater

distribution of small amounts of nutrients by many photosynthetic leaves when treated with BAP solutions so that leaves have smaller amounts of nutrients for shoot elongation (Arab et. al 86). Considering that other investigators conducted the experiment for at least 6 weeks, duration of the experiment could be a reason why the results of this experiment do not agree with that of others. Since BAP induces cell division, the length and width of leaves would be greater for plants treated with higher concentrations of BAP at least for the first growth period, as transgenic Arabidopsis overexpressed with genes coding for cytokinin oxidase showed a slower rate of leaf expansion compared to wild Arabidopsis plants (Werner. et al 10488).

## 5. Conclusion

Hypothesis A is partially supported by the results of this experiment. The average final number of leaves increased as the concentration of BAP increased upto 6 ppm. Above 6 ppm, the number of leaves decreased, suggesting that the leaf multiplication would be inhibited above the optimum concentration. In general, the average final widths and lengths of the longest leaves also increased with the concentration of BAP, displaying the induction of leaf expansion. However, if the experiment was conducted for a longer period, leaf expansion could have been smaller for *C.gladiata* treated with high BAP concentrations as suggested by other investigators (Arab et. al). $H_o$ is not rejected for the average final number of leaves and the averal final length of the longet leaves but rejected for the averal final width of the longest leaves.

To answer the research question, increasing BAP concentration showed inductive effects on the leaf growth for number, lengths and widths of leaves upto an optimum concentration, which was 6 ppm in this case, but showed inhibitory effects above 6 ppm in multiplicating leaves while high BAP concentrations were still favorable in leaf expansion.

# 6. Evaluation

## I. Experimental aspects

There are several limitations of this experiment. Firstly, the amount of sunlight that *C.gladiata* plants received could have differed to a considerable extent. Since first five trials were conducted in the late June to the mid-July and the last three trials were conducted in the mid-July to the early August, number of cloudy or rainy days could have been different. Therefore, plants could have received different amounts of sunlight and plants that received more sunlight could have shown a greater leaf growth, decreasing the accuracy of the results. In order to improve on this limitation, the experiment should be conducted at the same time for all trials to reduce such discrepancies - number of rainy or cloudy days and temperature in particular.

Secondly, the ages of C.*gladiata* beans are unknown, which could have decreased the accuracy of the results. The age of beans or the seed age is the amount of time that it stayed dormant after its harvest. Younger seeds show a greater rate in seedling growth than that of older seeds (Liu et. al). Thus, older *C.gladiata* beans could have shown a slower growth rate due to their age, making the correlation between the BAP concentration and the leaf growth of *C.gladiata* vague. When conducting the same experiment, bean with the same dormancy period should be used. Nurseries would provide such beans when conditions are explained.

Lastly, duration of the experiment was not substantial to acquire as much and insightful information as possible. As explained previously, BAP was not an appropriate hormone for leaf expansion according to several investigators, but higher concentrations of BAP resulted in a better leaf expansion in this experiment possibly due to a short duration of the experiment. A longer duration of the experiment, at least six weeks, could help proving the effects of BAP on leaf expansion of *C.gladiata* and analyzing how the effects of BAP change over time.

## II. Real life applications

Since the experiment was conducted indoors, it is unknown if the application of BAP would change how *C.gladiata* behaves to environmental pressures such as, drought. Since *C.gladiata* is a tropical plant, it is likely to suffer droughts in the dry seasons. It was found that wheat that was exogenously applied with BAP showed improved drought tolerance. In drought conditions, chlorophyll contents were increased for wheat treated with BAP, showing that BAP prevents reduction in essential metabolisms in plants such as, photosynthesis as a response to an environment pressure (Novakova et. al). In flood conditions, BAP has found to enhance leaf extension and prevent loss of leaves (Patel PK). These additional benefits of BAP increase the efficacy of using it as a fertilizer. However, as mentioned in 1.Introduction V.Safety and Ethics, BAP is hazardous to aquatic organisms. If BAP is sprayed to crops that grow near rivers, substantial amounts of BAP can permeate through the streams, leading to mass killing of aquatic organisms. Therefore, local regulation, liming the amount of BAP sprayed to crops grow near rivers, should be enforced to protect aquatic organisms.

Secondly, it is unclear that *C.gladiata* plants that showed the greatest leaf growth would produce the greatest crop yield. It was claimed by several scientists that multiple branching of axillary buds is linked with increased crop yield in monocotyledon rice plants (Yaish et. al 37) but it is uncertain that this finding will be also true for *C.gladiata*. Although the number of beans produced from *C.gladiata* plants is unknown, the number of leaves produced from *C.gladiata* plants treated with 6 ppm BAP solution is found to be the greatest. As mentioned in the Introduction, leaves of *C.gladiata* are consumed as tea so plants with more number and larger leaves will be commercially advantageous.

Despite the limitations, the investigation provided a valuable implication about the interaction between plant hormones and growth. It clearly showed that hormone homeostasis also occurs in plants just like animals and that excessive addition of hormone could negatively affect their growth. Therefore, this study provided insight that the concentration of cytokinins for either in *vitro* propagation of *C.gladiata* or improving the crop yield should be carefully selected to prevent negative consequences of hormone homeostasis.

# 7. Works Cited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# 8. Appendices

## I. Raw data

| |
|---|
| Indicates the first week of measurements |
| Indicates the second week of measurements |
| Indicates the third week of measurements |

| Day | Number of leaves | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Trial 1** | **Trial 2** | **Trial 3** | **Trial 4** | **Trial 5** | **Trial 6** | **Trial 7** | **Trial 8** |
| 0 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 2 | 5 | 5 | 2 | 2 | 2 | 2 | 2 | 2 |
| 5 | 6 | 5 | 3 | 2 | 2 | 5 | 2 | 5 |
| 7 | 6 | 5 | 4 | 2 | 2 | 5 | 5 | 5 |
| 9 | 7 | 5 | 5 | 3 | 2 | 5 | 5 | 5 |
| 12 | 7 | 5 | 5 | 3 | 2 | 8 | 8 | 5 |
| 14 | 7 | 9 | 5 | 3 | 2 | 9 | 8 | 8 |
| 16 | 10 | 9 | 8 | 6 | 2 | 9 | 10 | 10 |

**Table 8.1 - Number of leaves without BAP solution against time (arbitrary)**

| Day | Width of the longest leaf | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Trial 1** | **Trial 2** | **Trial 3** | **Trial 4** | **Trial 5** | **Trial 6** | **Trial 7** | **Trial 8** |
| 0 | 3.4 | 3.2 | 3.1 | 3.1 | 2.1 | 4.2 | 3.9 | 2.9 |
| 2 | 7.8 | 6.7 | 5.5 | 5.5 | 3.9 | 6.2 | 6.1 | 5.5 |
| 5 | 9.0 | 9.6 | 9.9 | 9.8 | 4.9 | 8.1 | 7.7 | 7.3 |
| 7 | 9.9 | 10.1 | 10.6 | 10.2 | 7.3 | 8.7 | 8.3 | 7.5 |
| 9 | 10.6 | 10.2 | 10.7 | 10.2 | 8.5 | 9.3 | 8.4 | 8.4 |
| 12 | 11.5 | 10.4 | 10.7 | 10.5 | 8.9 | 9.5 | 9.4 | 9.0 |
| 14 | 12.2 | 10.4 | 11.0 | 11.0 | 10.0 | 9.6 | 9.4 | 9.9 |
| 16 | 12.2 | 10.7 | 11.1 | 11.0 | 10.2 | 10.2 | 9.6 | 10.4 |

**Table 8.2 - Width of the longest leaf without BAP solution against time (±0.1cm)**

| Day | Length of the longest leaf | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Trial 1** | **Trial 2** | **Trial 3** | **Trial 4** | **Trial 5** | **Trial 6** | **Trial 7** | **Trial 8** |
| 0 | 4.2 | 3.5 | 4.1 | 3.6 | 2.5 | 4.6 | 4.7 | 3.7 |
| 2 | 8.6 | 7.3 | 6.2 | 5.8 | 4.1 | 7.4 | 6.8 | 6.4 |
| 5 | 12.0 | 11.4 | 10.5 | 11.3 | 4.3 | 9.1 | 8.3 | 8.2 |
| 7 | 12.3 | 11.5 | 11.9 | 12.0 | 4.6 | 10.2 | 9.8 | 9.5 |
| 9 | 12.5 | 12.3 | 13.4 | 12.5 | 6.0 | 11.3 | 10.5 | 10.2 |
| 12 | 13.1 | 12.4 | 14.0 | 13.3 | 7.0 | 11.7 | 11.1 | 10.9 |
| 14 | 13.1 | 13.0 | 14.0 | 13.5 | 7.5 | 11.9 | 11.3 | 11.4 |
| 16 | 13.3 | 13.0 | 14.3 | 13.7 | 7.8 | 12.2 | 12.0 | 11.7 |

**Table 8.3 - Length of the longest leaf without BAP solution against time (±0.1cm)**

| Day | Number of leaves | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Trial 1** | **Trial 2** | **Trial 3** | **Trial 4** | **Trial 5** | **Trial 6** | **Trial 7** | **Trial 8** |
| 0 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 2 | 2 | 5 | 4 | 2 | 2 | 2 | 2 | 5 |
| 5 | 2 | 5 | 4 | 2 | 6 | 5 | 5 | 5 |
| 7 | 2 | 5 | 4 | 2 | 6 | 5 | 5 | 5 |
| 9 | 2 | 7 | 7 | 2 | 9 | 5 | 8 | 8 |
| 12 | 2 | 7 | 7 | 2 | 9 | 8 | 8 | 10 |
| 14 | 2 | 7 | 7 | 5 | 9 | 9 | 8 | 10 |
| 16 | 5 | 10 | 7 | 8 | 9 | 9 | 9 | 10 |

**Table 8.4 - Number of leaves for 2 ppm BAP solution against time (arbitrary)**

| Day | Width of the longest leaf | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Trial 1** | **Trial 2** | **Trial 3** | **Trial 4** | **Trial 5** | **Trial 6** | **Trial 7** | **Trial 8** |
| 0 | 1.6 | 4.1 | 4.3 | 2.9 | 3.1 | 3.5 | 2.9 | 3.1 |
| 2 | 5.3 | 5.8 | 6.4 | 4.2 | 3.7 | 5.4 | 3.8 | 4.0 |
| 5 | 10.6 | 8.1 | 9.4 | 5.6 | 6.9 | 5.9 | 4.9 | 4.7 |
| 7 | 12.0 | 8.7 | 10.4 | 6.8 | 8.0 | 6.1 | 5.7 | 5.4 |
| 9 | 13.5 | 9.4 | 11.9 | 7.6 | 11.1 | 6.5 | 6.2 | 6.0 |
| 12 | 14.7 | 9.6 | 12.2 | 8.0 | 11.2 | 9.6 | 8.9 | 9.1 |
| 14 | 16.0 | 9.8 | 13.0 | 10.1 | 12.5 | 9.7 | 9.0 | 9.3 |
| 16 | 16.1 | 9.9 | 13.0 | 10.2 | 12.7 | 10.4 | 10.5 | 9.7 |

**Table 8.5 - Width of the longest leaf for 2 ppm BAP solution against time (±0.1cm)**

| Day | Length of the longest leaf | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Trial 1** | **Trial 2** | **Trial 3** | **Trial 4** | **Trial 5** | **Trial 6** | **Trial 7** | **Trial 8** |
| 0 | 2.8 | 4.8 | 5.6 | 2.7 | 2.9 | 4.1 | 3.5 | 3.7 |
| 2 | 5.2 | 6.3 | 6.5 | 3.5 | 3.3 | 6.0 | 4.2 | 4.5 |
| 5 | 9.1 | 6.6 | 10.0 | 4.7 | 6.9 | 6.4 | 5.8 | 5.4 |
| 7 | 10.1 | 7.0 | 10.1 | 5.8 | 7.4 | 6.6 | 6.2 | 5.9 |
| 9 | 12.4 | 7.2 | 12.5 | 6.5 | 10.5 | 7.2 | 6.9 | 6.4 |
| 12 | 12.6 | 7.6 | 12.5 | 6.6 | 10.9 | 10.3 | 9.4 | 9.3 |
| 14 | 13.3 | 7.6 | 12.5 | 8.4 | 11.5 | 10.6 | 10.2 | 10.0 |
| 16 | 13.4 | 8.3 | 12.8 | 8.6 | 11.7 | 11.8 | 11.0 | 10.5 |

**Table 8.6 - Length of the longest leaf for 2 ppm BAP solution against time (±0.1cm)**

| Day | Number of leaves | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Trial 1** | **Trial 2** | **Trial 3** | **Trial 4** | **Trial 5** | **Trial 6** | **Trial 7** | **Trial 8** |
| 0 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 2 | 4 | 5 | 2 | 2 | 2 | 5 | 4 | 5 |
| 5 | 4 | 6 | 2 | 2 | 2 | 5 | 5 | 5 |
| 7 | 4 | 6 | 2 | 2 | 2 | 7 | 5 | 7 |
| 9 | 7 | 9 | 2 | 2 | 5 | 7 | 8 | 7 |
| 12 | 7 | 9 | 5 | 2 | 5 | 8 | 8 | 9 |
| 14 | 7 | 9 | 5 | 8 | 5 | 9 | 9 | 9 |
| 16 | 10 | 9 | 8 | 8 | 8 | 10 | 9 | 9 |

**Table 8.7 - Number of leaves for 4 ppm BAP solution against time (arbitrary)**

| Day | Width of the longest leaf | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Trial 1** | **Trial 2** | **Trial 3** | **Trial 4** | **Trial 5** | **Trial 6** | **Trial 7** | **Trial 8** |
| 0 | 3.1 | 4.4 | 4.5 | 4.9 | 3.9 | 2.8 | 3.7 | 3.5 |
| 2 | 5.0 | 6.2 | 6.8 | 6.2 | 9.1 | 6.2 | 5.5 | 6.9 |
| 5 | 10.7 | 10.6 | 12.4 | 7.3 | 13.1 | 7.4 | 6.7 | 7.5 |
| 7 | 11.1 | 10.6 | 13.0 | 7.5 | 13.5 | 8.5 | 8.5 | 8.1 |
| 9 | 12.0 | 11.5 | 13.6 | 8.2 | 13.5 | 9.3 | 9.1 | 9.7 |
| 12 | 12.4 | 12.0 | 13.7 | 9.6 | 13.6 | 10.6 | 9.9 | 11.5 |
| 14 | 14.5 | 12.5 | 13.8 | 10.4 | 14.0 | 12.8 | 10.7 | 12.2 |
| 16 | 14.5 | 12.6 | 13.9 | 10.8 | 14.2 | 14.7 | 11.8 | 13.2 |

**Table 8.8 - Width of the longest leaf for 4 ppm BAP solution against time (±0.1cm)**

| Day | Length of the longest leaf | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Trial 1** | **Trial 2** | **Trial 3** | **Trial 4** | **Trial 5** | **Trial 6** | **Trial 7** | **Trial 8** |
| 0 | 3.2 | 4.3 | 3.8 | 6.5 | 4.3 | 3.1 | 4.5 | 3.7 |
| 2 | 6.0 | 6.8 | 6.6 | 7.3 | 8.7 | 6.3 | 6.7 | 6.5 |
| 5 | 11.5 | 11.4 | 11.4 | 8.2 | 13.5 | 9.8 | 7.1 | 9.5 |
| 7 | 12.1 | 11.7 | 12.5 | 8.6 | 14.0 | 12.5 | 7.8 | 10.7 |
| 9 | 14.3 | 12.0 | 14.6 | 9.2 | 14.3 | 13.5 | 8.5 | 11.3 |
| 12 | 14.4 | 12.0 | 15.2 | 10.5 | 14.4 | 14.1 | 10.2 | 12.1 |
| 14 | 14.6 | 12.5 | 15.2 | 11.0 | 14.5 | 14.7 | 11.3 | 12.8 |
| 16 | 14.6 | 12.7 | 15.6 | 11.3 | 14.7 | 15.1 | 12.1 | 13.4 |

**Table 8.9 - Length of the longest leaf for 4 ppm BAP solution against time (±0.1cm)**

| Day | Number of leaves | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Trial 1** | **Trial 2** | **Trial 3** | **Trial 4** | **Trial 5** | **Trial 6** | **Trial 7** | **Trial 8** |
| 0 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 2 | 2 | 4 | 4 | 2 | 4 | 2 | 3 | 2 |
| 5 | 2 | 4 | 4 | 2 | 4 | 2 | 5 | 5 |
| 7 | 2 | 4 | 4 | 2 | 4 | 5 | 5 | 7 |
| 9 | 5 | 7 | 7 | 5 | 7 | 5 | 7 | 9 |
| 12 | 8 | 8 | 7 | 5 | 7 | 7 | 7 | 9 |
| 14 | 8 | 8 | 11 | 5 | 8 | 8 | 10 | 10 |
| 16 | 11 | 11 | 11 | 8 | 11 | 8 | 10 | 10 |

**Table 8.10 - Number of leaves for 6 ppm BAP solution against time (arbitrary)**

| Day | Width of the longest leaf | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Trial 1** | **Trial 2** | **Trial 3** | **Trial 4** | **Trial 5** | **Trial 6** | **Trial 7** | **Trial 8** |
| 0 | 3.5 | 3.2 | 3.0 | 3.4 | 2.6 | 2.4 | 3.2 | 3.7 |
| 2 | 7.7 | 7.4 | 6.6 | 7.9 | 5.9 | 5.3 | 7.1 | 7.5 |
| 5 | 11.3 | 9.9 | 11.1 | 9.9 | 10.5 | 6.2 | 8.6 | 8.0 |
| 7 | 11.6 | 13.0 | 11.5 | 10.5 | 12.1 | 6.9 | 9.5 | 8.7 |
| 9 | 11.7 | 13.5 | 13.0 | 10.7 | 13.2 | 7.6 | 10.6 | 9.4 |
| 12 | 12.0 | 13.5 | 13.5 | 11.0 | 13.3 | 9.1 | 11.3 | 10.2 |
| 14 | 12.6 | 13.6 | 13.7 | 11.3 | 13.4 | 10.5 | 12.1 | 11.6 |
| 16 | 12.6 | 13.6 | 13.9 | 11.4 | 13.6 | 11.2 | 12.9 | 13.8 |

**Table 8.11 - Width of the longest leaf for 6 ppm BAP solution against time (±0.1cm)**

| Day | Length of the longest leaf | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Trial 1** | **Trial 2** | **Trial 3** | **Trial 4** | **Trial 5** | **Trial 6** | **Trial 7** | **Trial 8** |
| 0 | 3.7 | 3.4 | 2.9 | 3.5 | 2.8 | 2.8 | 3.4 | 3.8 |
| 2 | 8.3 | 7.6 | 7.7 | 9.5 | 6.3 | 5.9 | 7.5 | 8.0 |
| 5 | 13.1 | 11.7 | 12.3 | 13.4 | 13.5 | 6.4 | 9.2 | 8.5 |
| 7 | 13.6 | 12.5 | 12.5 | 13.5 | 14.2 | 7.2 | 10.1 | 9.2 |
| 9 | 14.0 | 12.7 | 12.7 | 13.6 | 15.0 | 8.1 | 11.2 | 10.1 |
| 12 | 14.0 | 13.5 | 13.0 | 14.0 | 15.5 | 9.9 | 12.0 | 10.9 |
| 14 | 14.5 | 13.5 | 13.0 | 14.5 | 15.6 | 10.9 | 12.6 | 12.4 |
| 16 | 14.5 | 13.6 | 13.3 | 14.6 | 15.8 | 13.2 | 13.5 | 14.2 |

**Table 8.12 – Length of the longest leaf for 6 ppm BAP solution against time (±0.1cm)**

| Day | Number of leaves | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Trial 1** | **Trial 2** | **Trial 3** | **Trial 4** | **Trial 5** | **Trial 6** | **Trial 7** | **Trial 8** |
| 0 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 2 | 2 | 4 | 2 | 2 | 2 | 2 | 2 | 2 |
| 5 | 4 | 5 | 2 | 2 | 2 | 2 | 2 | 5 |
| 7 | 4 | 5 | 2 | 2 | 2 | 5 | 5 | 5 |
| 9 | 7 | 5 | 8 | 2 | 5 | 5 | 5 | 5 |
| 12 | 7 | 5 | 9 | 2 | 5 | 7 | 5 | 8 |
| 14 | 7 | 5 | 9 | 3 | 7 | 7 | 7 | 8 |
| 16 | 9 | 8 | 9 | 3 | 7 | 8 | 7 | 8 |

**Table 8.13 - Number of leaves for 8 ppm BAP solution against time (arbitrary)**

| Day | Width of the longest leaf | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Trial 1** | **Trial 2** | **Trial 3** | **Trial 4** | **Trial 5** | **Trial 6** | **Trial 7** | **Trial 8** |
| 0 | 3.2 | 2.9 | 2.8 | - | 3.2 | 3.2 | 2.5 | 3.3 |
| 2 | 6.7 | 7.0 | 3.3 | - | 7.5 | 6.4 | 6.9 | 6.2 |
| 5 | 12.1 | 8.4 | 11.0 | - | 11.9 | 8.1 | 10.4 | 9.8 |
| 7 | 12.4 | 10.0 | 11.1 | - | 12.0 | 10.4 | 11.0 | 10.9 |
| 9 | 12.5 | 11.1 | 11.3 | - | 12.4 | 11.2 | 12.1 | 11.6 |
| 12 | 12.6 | 13.0 | 11.6 | - | 15.7 | 11.3 | 13.3 | 12.1 |
| 14 | 12.6 | 13.1 | 12.0 | - | 15.8 | 12.4 | 13.7 | 12.9 |
| 16 | 12.9 | 13.3 | 12.4 | - | 16.5 | 13.0 | 13.8 | 13.5 |

**Table 8.14 - Width of the longest leaf for 8 ppm BAP solution against time (±0.1cm)**

*** - means that measurable leaves were not formed***

| Day | Length of the longest leaf | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Trial 1** | **Trial 2** | **Trial 3** | **Trial 4** | **Trial 5** | **Trial 6** | **Trial 7** | **Trial 8** |
| 0 | 3.3 | 3.2 | 3.2 | - | 3.6 | 3.4 | 3.1 | 3.5 |
| 2 | 8.0 | 6.9 | 3.7 | - | 7.6 | 6.7 | 7.3 | 6.9 |
| 5 | 12.5 | 8.5 | 12.5 | - | 13.3 | 8.4 | 10.8 | 10.2 |
| 7 | 13.0 | 9.5 | 12.6 | - | 14.0 | 10.5 | 11.5 | 11.4 |
| 9 | 14.0 | 12.5 | 13.1 | - | 14.2 | 11.4 | 12.8 | 12.0 |
| 12 | 14.0 | 15.4 | 13.2 | - | 15.0 | 12.0 | 13.6 | 12.9 |
| 14 | 14.2 | 15.5 | 13.4 | - | 15.1 | 12.8 | 14.0 | 13.2 |
| 16 | 14.4 | 15.5 | 13.7 | - | 15.5 | 13.5 | 14.1 | 13.8 |

**Table 8.15 – Length of the longest leaf for 8 ppm BAP solution against time (±0.1cm)**

## II. Pictures of materials


**Picture 8.1 - *Canavalia gladiata***


**Picture 8.2 - 6-Benzylaminopurine (99.0%)**

## III. Pictures of *Canavalia gladiata* during the experiment

**Picture 8.3 - Hydration of *C.gladiata***


**Picture 8.4 - Growth of *C.gladiata***

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# Extended essay - Reflections on planning and progress form

***Candidate:*** *This form is to be completed by the candidate during the course and completion of their EE. This document records reflections on your planning and progress, and the nature of your discussions with your supervisor. You must undertake three formal reflection sessions with your supervisor: The first formal reflection session should focus on your initial ideas and how you plan to undertake your research; the interim reflection session is once a significant amount of your research has been completed, and the final session will be in the form of a viva voce once you have completed and handed in your EE. This document acts as a record in supporting the authenticity of your work. The three reflections combined must amount to no more than 500 words.*

**The completion of this form is a mandatory requirement of the EE for first assessment May 2018. It must be submitted together with the completed EE for assessment under Criterion E.**

***Supervisor:*** *You must have three reflection sessions with each candidate, one early on in the process, an interim meeting and then the final viva voce. Other check-in sessions are permitted but do not need to be recorded on this sheet. After each reflection session candidates must record their reflections and as the supervisor you must sign and date this form.*

## First reflection session

Candidate comments:

One of my friends has lactose intolerance and experienced lactose intolerance symptoms after she took five tablets of her cold medication. The pharmacist once warned her that she might experience stomachache, if she is lactose intolerant, after taking that medication. My friend's story triggered my interest in lactose content in medications. Therefore, I decided to research on how lactose in medications influence lactose intolerance patients but I realized that my research would be too obvious and general. Therefore, I narrowed down my research question: "To what extent does lactose content in commonly bought medications influence lactose-intolerance patients?" Then I added the second part to it "which substance could be an alternative of lactose?" to extend on the research on the first part of the question.

Date: March 6, 2018

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## Interim reflection

Candidate comments:

I changed my topic and research question due to difficulties in conducting an experiment. Either enzymatic assays and High Performance Liquid Chromatography required high technology equipment which were not accessible at school. My topic has changed to plant biology with the research question: “How does different concentrations of 6-Benzylaminopurine influence the leaf growth of Canavalia gladiator?”. I conducted the experiment with eight trials for each concentration and analysed the results by comparing mine with the results of other similar investigations. I finished my first draft and received feedback from my supervisor. The most crucial feedback I got was to linking back my research to real life at the end of the essay. I will make the real-life applications clearer by evaluating the drought and flood tolerance with the application of BAP and how BAP interacts with soil. The second most crucial feedback was safety so I have to research on the safety issues related to BAP especially on humans.

Date: September 21, 2018

## Final reflection - Viva voce

Candidate comments:

I have gained a lot of biological knowledge and research skills by writing an extended essay. Firstly, I have learned that adding too high concentration or too much plant hormones would lead to negative consequences due to hormone homeostasis. Secondly, I have learned that the growth of plants cannot be anticipated as plants are affected by many environmental factors - sunlight, water and humidity (transpiration). In terms of research skills, I learned that it is always good to have as many trials as possible especially for experiments using plants. Although I conducted eight trials for each concentration, eight trials were not enough to provide me enough evidence to reject the null hypothesis according to the T-test for correlation. I also learned that it is always beneficial to seek for help to my supervisors or other science teachers as they have solid knowledge on writing an extended essay, as part of their university assessments.

Date: December 15, 2018