Effect of different concentrations of vitamin c

This project was designed to investigate the effect of different concentrations of vitamin C solution on seed germination of Brassica parachinensis or Choy Sum. Seeds treated with vitamin C solutions of various concentrations were used and germination percentage was calculated. Seeds were soaked in vitamin C solutions of increasing concentrations for 12 hours, dried and then sown in germinating trays. The seeds were watered with the same amount of vitamin C solution of respective concentrations. After 36 hours, the number of germinated seeds was counted and the germination percentage was calculated and recorded. Results showed that the germination percentage increases as low concentration of vitamin C solution was used. It was shown that 1% of vitamin C solution gave the highest germination percentage. A statistical analysis using Pearson product-moment correlation coefficient showed a statistically significant negative linear relationship between concentration of vitamin C and germination percentage since the calculated r value was greater than critical value at 5% significant level.

Introduction

In order to increase productivity, it is important to improve overall growth and performance of agricultural crops. Germination rate in particular is crucial because an increase in germination rate is usually followed by an improvement of overall seedling performance thus enhancing overall plant growth and productivity.(Parera and Cantliffe, 1991).

There are many factors affecting seed germination such as temperature, humidity, presence of light and seed dormancy. Recently, there is also evidence to support a link between certain biochemical characteristics and

seed vigour.(Randhir and Shetty, 2003) Fast oxidation of fatty acids and proteins can reduce viability, vigour and germination percentage in seeds. 11

Vitamin C or ascorbic acid is one of the most widely taken dietary supplement. 5 It is an antioxidant which is an essential nutrient for living organisms as it protects the body against oxidative stress. Vitamin C is made naturally in plants but the amount depends on the variety of plant and external factors on which the plants grow. 1

Ascorbic acid is an important metabolite involved in many cellular processes, including cell division (De Gara et al., 2003). Ascorbate has been shown to play multiple roles in plant growth, such as in cell division, cell wall expansion, and other developmental processes. It can act as a coenzyme for numerous metabolic enzymes and has recently been shown to be a potent antioxidant. 12

Figure 1: The oxidation of ascorbic acid to dehydroascorbic acid 6

(410 words)

Vitamin C interacts with oxidants in an oxidation reaction where ascorbic acid is oxidised to dehydroascorbic acid through an ascorbyl radical intermediate. 8 The oxidized forms of ascorbate are relatively unreactive, and do not cause cellular damage. They can be converted back to ascorbate by cellular enzymes. 4 Application of ascorbic acid exogenously may influence various processes in plants including seed germination, ion uptake and transport, and membrane permeability. 12

Structure of vitamin C or L-ascorbic acid or Dehydroascorbic acid

L-ascorbate 4 (nominal oxidized form of Vitamin C) 4

Previous reports show some conflicting evidence about the effect of vitamin C towards seed germination. While some researchers say that vitamin C can promote seed vigour as in pea (Pisum Sativum) seeds, 12 some state that its presence will inhibit seed germination as in soybean seed. 13

Defined in the qualitative term, seed vigour is the sum of those properties of the seed which determine the potential level of activity and performance of the seed or seed lot during germination and seedling emergence. 12

Seeds of genus Brassica are used in this experiment because the seeds are small and have a short life cycle. Therefore, experiment can be done within few days and the seeds can be used in large number with less space needed.

Hence, this experiment was aimed to explore the benefits of vitamin C which is an antioxidant which may help to increase plant resistance that leads to enhanced crops.

(647 words)

Experimental Hypothesis

The lower the concentration of vitamin C solution, the higher the germination percentage of Brassica parachinensis

Null Hypothesis

There is no correlation between different concentrations of vitamin C solutions and the germination percentage of Brassica parachinensis

(685 words)

Planning

Trials

Trial experiment was conducted to determine the best range of concentrations of vitamin C solution to be used and the reasonable methods and procedures so that good results are obtained.

Methods Involved

Preparing vitamin C solution of different concentrations

Since the source of vitamin C used is the vitamin C tablet, the best method chosen is the serial dilation method. During trial, concentrations tested are 100%, 75%, 50%, 25%, 10% and 0%. The basic stock solution of 100% was prepared by dissolving 100mg of vitamin C tablet in 100ml distilled water in a 300ml beaker. Other concentrations were made by serial dilution method. To prepare 50% concentration of vitamin C solution, same amount of 100% solution and distilled water were added together. Equal amount of 100% and 50% concentrations were mixed up to make 75% concentration and so on.

Sowing the seeds

The seeds were primed in 6 different Petri dishes for 12 hours according to its respective concentration. Another 6 Petri dishes were labelled. Layers of cotton of the same thickness were put into Petri dishes and moisten with the same volume of vitamin C solution of different concentrations. Soaked seeds with similar size were chosen and took out of Petri dish using a pair of

forceps. They are then dried using paper towels and were sown in labelled Petri dishes.

(909 words)

In the first trial, 25 seeds were soaked in different concentrations of vitamin C solution while another 25 seeds were not soaked before being sown. This is to determine whether it is necessary to soak seeds or not before sowing.

36 hours after sowing seeds

Concentration/%

Number of seeds germinated

Soaked seeds

0

15

10

16

50

1

Table 1 shows the number of soaked and unsoaked seeds after 36 hours

In the second trial, each group of 25 seeds were soaked in different concentrations before being sown. This is to determine the best range of concentrations of vitamin C for main experiment.

36 hours after sowing seeds

Concentration/%

Number of seed germinated

Trial 1

Trial 2

Mean

0

15

16

16

10

18

16

17

25

5

5

5

50

1

3

2

75

2

1

2

100

1

0

1

Table 2 shows germination percentage of seeds soaked in different concentrations

(1075 words)

Results

After 36 hours of sowing, the number of seeds germinated was counted and germination percentage was calculated. Germination is claimed to have occurred when the radicle was visibly extended from the seed, protruding through a fracture in the seed coat. The result was tabulated.

Based on the results obtained after trials were carried out, there were a few things that could be done to improve result. Firstly, more seeds should be used so that the result will be more significant. For the real experiment, 50 seeds were used instead of 25 seeds.

Seeds were primed for 12 hours because priming is important as seed coat contains some chemicals that inhibit germination. Seed priming is a common agronomic technique shown to cause an increase seed vigour, as well as synchronize and accelerate germination, improve stress resistance, and enhance overall plant growth and productivity. 12 However, some of the seeds start to germinate and fractures on seed coat could be observed if the seeds were primed for too long.

Besides, the range of concentration to be used in real experiment was determined to be between 0% to 10% concentrations because both showed high percentage of seed germination with only slight difference in value.

Seeds soaked in 100% concentration showed the lowest germination percentage. The germination of seeds was almost totally inhibited in this solution. Fractures on the seed coats were observed but radicles were not extended significantly.

Besides, after carrying out trial experiment, I realized that it was better to sow seeds in germinating tray rather than in Petri dishes as Petri dish is round-shaped which made it difficult for me to count the number of seeds germinated.

(1351 words)

Main experiment

Variables

Manipulated: Concentration of vitamin C solution

Different concentrations of vitamin C are prepared using the same serial dilution technique as in trials. But this time using smaller range of concentrations of 10%, 7. 5%, 5%, 2. 5%, 1% and 0% (distilled water). But for smaller concentration, micropipette is used instead of measuring cylinder to make up the total volume of 100ml.

Responding: Germination percentage (%)

Germination percentage is calculated using the following equation;

Germination is said to occur when radicle emerges (> 2mm) after 36 hours.

Constant: mass of vitamin C (100mg/tablet), volume of solution used for soaking seeds (20ml/Petri dish) and volume of solution used to moisten cotton (60ml/tray) and environmental factors (light intensity, temperature, and humidity), period of soaking, type and number of seeds. All trays were placed under normal white fluorescent lamp and on a laboratory table at 28ËšC.

Apparatus and materials

Apparatus:

300ml beakers, Petri dishes, measuring cylinders, germinating trays, stirring rod, micropipette, and pestle and mortar.

Materials:

Distilled water, 100mg vitamin C tablet, cotton, choy sum seeds, and paper towels.

(1535 words)

Safety precautions

Gloves were worn when handling vitamin C tablet to prevent tablet from contamination.

Micropipette was used with care. The tip of micropipette was always checked to ensure that there is no air bubbles inside it as this may reduce the actual volume required for each concentration.

Glass wares like beakers and measuring cylinders were handled gently since the apparatus may break easily. This may lead to injuries.

Soaked seeds were transferred gently using forceps as they are very fragile.

Data collections of main study

Number of seeds used in every concentration: 50

Soaking for 12 hours

Observations after soaking seeds for 36 hours

Concentration (%)

Number of seeds germinated

Mean number of seeds germinated

Replicate 1

Replicate 2

Replicate 3

0. 0

45

44

43

44

1. 0

48

48

47

48

2. 5

42

39

38

40

5. 0

41

42

43

42

7. 5

35

35

34

35

10. 0

34

33

33

33

Table 2 shows the germination percentage for seeds treated with different concentrations

(1712 words)

Sample calculation (taken values from 0% concentration)

Germination percentage:

= 44/50 x 100

= 88%

(1749 words)

Statistical analysis

For this experiment, Pearson product-moment correlation coefficient (PMCC) was used to measure the strength of linear dependence between the two variables; the different concentrations and the germination percentage. The values obtained are normally distributed.

The correlation coefficient, r ranges from -1 to 1. A value of 1 implies that a linear equation describes the relationship between X and Y perfectly for which Y increases as X increases. A value -1 implies correlation in which Y

decreases as X increases. A value of 0 implies that there is no linear correlation between the variables.

x

0. 0

1. 0

2. 5

5. 0

7. 5

10. 0

Σx= 26

y

88

96

80

84

70

66

Σy= 484

x2

0. 0

1. 00

6. 25

25. 0

56. 25

100. 0

Σx2= 188. 5

y2

7744

9216

6400

7056

4900

4356

Σy2= 39672

xy

0. 0

96

200

420

525

660

Σxy= 1901

Table 3 shows the calculations done in calculating the values of r

Sx = x2 –

= 188. 5 – (26)2/6

= 75. 833

Sy = y2 –

= 39672 – (484)2/6

= 629. 33

Sxy = xy –

= 1901 – [(26) (484)]/6

= -196. 33 (1933 words)

To find correlation coefficient, r

r =

r = -196. 33/ (75. 8331/2 x 629. 331/2)

r = -0. 899 (negative sign indicates negative correlation between variables)

Degree of freedom = n -2

n = # of pairs of data

= 6 – 2 = 4

Level of significance for two-tailed test

. 10

. 05

. 02

1

. 988

. 997

. 9995

2

. 900

. 950

. 980

3

. 805

. 878

. 934

4

. 729

. 811

. 882

5

. 669

. 754

. 833

6

. 621

. 707

. 789

Table 4 shows the critical values for PMCC

The value of r obtained was compared with the critical value from Table 4 at 5% significance level.

r = 0. 899 > 0. 811 (critical value) at 5% significance level

An analysis using PMCC demonstrated a statistically significant negative linear relationship between concentration of vitamin C and germination percentage since calculated r value was greater than the critical value at 5% significance level.

Therefore, null hypothesis can be rejected.

(2093 words)

Data analysis

Based on the data collected in Table 2, it was shown that low concentration of vitamin C did promote germination of choy sum to a certain extent. The statistical analysis using PMCC further verified the correlation. The table also revealed that only low concentration of vitamin C was needed to hasten germination since under 10% concentration, seeds germinated at a lower rate (66%).

Graph 1 illustrates clearly the general trend and relationship between the variables, the lower the concentration of vitamin C, the lower the germination rate of choy sum. The highest germination rate (96%) was recorded by seeds sown in 1% vitamin C concentration which was the lowest concentration used.

However, it can be seen that the line is fluctuating downwards and there is a linear graph showing that the values obtained were due to certain errors that lead to some anomalous results. At 5% concentration of vitamin C, we can see that the germination rate is slightly higher than that of 2. 5% but it is not supposed to be so.

Some of errors that may occur during experiment are

Mixing error when preparing solutions of different concentrations by serial dilution.

The thickness of cotton layer in germinating tray may not be constant throughout the tray so vitamin C solution with thinner cotton layer may get evaporated faster thus absorption of solution is less for the seed in that tray

Some of seeds may have been initially damaged so germination may not happen

(2342 words)

The germination percentage was compared with the result from other published academic journals. The germination percentage against day graph below shows the results from journal titled Effect of vitamin C and folic acid on seed vigour response and phenolic-linked antioxidant activity from source 12. It shows that the optimum concentration of vitamin C for pea seed to germinate is 500micromol/dm3.

Using appropriate calculations, it can be calculated that 10% concentration is approximately equal to 500micromol/dm3. 9 Thus, the germination

percentage of pea seed in 10% concentration is highest compared to other concentrations. However, the result I obtained for highest germination percentage for Brassica parachinensis was different. This can be due to some errors and limitations occurred during experiment or the fact that they are of different types of seed which is further discussed in limitation and modification part.

In this experiment, the constant or controlled variable is the use of distilled water (0% concentration). Since the results showed that the difference between germination rate between 0% and 1% concentration was very small (only about 8%), this indicated that the effect of vitamin C on seed vigour may be too little. Even though the effect is little, it cannot be denied that this little help from vitamin C can give a huge impact on agricultural sector as it is able to promote seed germination therefore improving mass production of plants rather than watering seeds with water only.

(2577 words)

Evaluation

Seeds are exposed to various biotic and abiotic stresses during germination. Oxidative stress for example, caused by the production of high level of reactive oxygen intermediates such as O2, O2-, H2O2, and HO- can lead to the destruction of cells. 4 Presence of low concentration of exogenous vitamin C stimulates seed vigour as it minimises oxidative stress by scavenging these reactive oxygen species.

Many oxidants contain an unpaired electron so they are highly reactive. These free radicals are damaging because they can interact with other

molecules resulting in a whole chain of free radical reactions. Ascorbate can terminate these chained radical reactions by serving as a stable (electron + proton) donor in interactions with free radicals. 4

However, high concentration of vitamin C solution inhibits seed germination because as solution becomes more concentrated, more water from cells diffuse out causing cellular membrane leakage. 3

Besides that, being a good electron donor, excess ascorbate in the presence of free metal ions can not only promote, but also initiate free radical reactions, thus making it a potentially dangerous pro-oxidative compound in certain metabolic contexts. 8 We hypothesize that whether vitamin C has a net pro oxidant or antioxidant effect depends on the concentration of vitamin C solution. 8

With relation to improving seed vigour through stimulation of phenolic synthesis, it has been suggested that exogenously applied phenolic antioxidants may be able to stimulate endogenous phenolic content in plants (Randhir and Shetty, 2003 and Randhir et al., 2002).

Due to its inherent antioxidant potential, vitamin C seems to be a suitable growth regulator in increasing seed vigour and phenolic elicitation. In this study, we have hypothesized that the treatment of plants with vitamin C as exogenous seed treatments may result in an increase in both biochemical and traditional agronomic indicators of seed vigour. 11

(2873 words)

Limitations and modifications

One of the limitations in this experiment is the changing surrounding temperature. The seeds were left in laboratory room where the temperature may fluctuate over time. This may affect the germinating time of the seeds and the number of germinated seed after 36 hours. Besides temperature, humidity and light intensity were also uncontrolled. Since all seeds were exposed to the same environmental factors, the result is considered to be reliable.

Besides that, some seeds may have been initially damaged before soaking or sowing. Therefore, vitamin C solution may not be the actual cause of inhibition of seed germination. The seeds are chosen only by physical measures such as similarity in size, shape and colour and seed coat is not broken.

However, limitations can be overcome by using a large number of seeds and the experiment is repeated for a few times to ensure that result obtained is reliable and enough to support conclusion. This investigation tests only on the effectiveness of vitamin C solution on seed germination without exposing seeds to other biotic stress such as salinity or abiotic stress such as exposure to sunlight.

Modifications could be made by introducing a biotic or abiotic stress to the seed and observe if vitamin C still stimulates seed germination or otherwise. Different types of seed may have different result with vitamin C solution. So, using seeds from one type of plant may not be able to indicate how vitamin C actually acts on seed. As vitamin C may enhance seed vigour in certain

species, it could do otherwise to other species. Hence, the same experiment should be done to seeds from other species to see how they are affected by vitamin C solution.

Conclusion

The lower the concentration of vitamin C, the higher the germination rate of Brassica parachinensis seeds. This is due to the antioxidant properties of vitamin C which acts against the oxidative stress. The optimum vitamin C concentration for seed germination is 1%.

(3200 words)

Source Evaluation

I have referred to various sources in conducting my research.

Sources 1, 2, 3 and 4 are from Wikipedia which has been the largest and most popular general reference work on the internet. Although many have doubts over its reliability as it can be edited by anyone with access to the site but it is cited and linked to many other literatures and academic researches around the globe. Source 5 is a website written by health experts on health and medicine. Source 6 is a website that provides information written by university scholars with reference to other academic researches. Source 7 is reliable as it is written by Dr. Bryan D. McKersie of University of Guelph and is posted on the internet in 1996. Source 8 is a reliable source as it is an article by The American Society for Nutritional Sciences and was published in The Journal of Nutrition website in 2004. Source 9 has been a referral site to many people around the world. The information seems reliable as it is written by experts.

Source 10 is a published book thus the information is reliable.

Source 11 is a journal from ScienceDirect which I have accessed from my campus library. ScienceDirect is a leading full-text scientific database offering journal articles and book chapters from more than 2, 500 peer-reviewed journals and more than 11, 000 books. There are currently more than 9. 5 million articles/chapters, a content base that is growing at a rate of almost 0. 5 million additions per year. It is operated by the publisher Elsevier. Source 12 is a journal advised by an international Editorial Board and is abstracted or indexed by various research databases like the EBSCO and Elsevier. Source 13 is an article from the Journal of Biological Chemistry.