CHAPTER 3

SEED GERMINATION

3.1 INTRODUCTION

The seed constitutes the main vector of plant propagation. *Hylocereus* fruit contains thousand of seeds which have testa, endosperm and embryo (Rojas-arehchiga and Vahzquez-yanes, 2000). Germination starts with the uptake of water through imbibitions of dry seed followed by embryo expansion and usually the radical extends to penetrate the structures that surround it (Mei and Song, 2008 and Bewley, 1997). Seed dormancy can be defined as the failure of an intact viable seed to germinate even when the condition favours germination. Seed dormancy has a survival value when the conditions are not favourable for germination (Koornneef *et al.*, 2002 and Rojas-arehchiga and Vahzquez-yanes C, 2000).

The most common cultivating technique for dragon fruit is stem cutting where the stem is removed and tied onto a supportive material for it to grow. The supporting material can be man-made stilts or consists of dead organic material such as tree bark or a living tree which provides the support. Stem cutting is the preferred way as it can produce fruit rapidly in a year compared to seed germination which takes 3 years to bear fruits (Le Bellec *et al.*, 2006). Due to vegetative propagation, there is no natural variation and genetic uniformity in crops is undesirable as it tends to make the crop vulnerable to epidemics and environmental disasters resulting in yield loss. Although seed germination takes a relatively longer time for the plant to produce fruit as compared to stem cutting, seed germination is useful in providing the starting material for DNA extraction. As the fruit contains thousand of seeds,

seeds germination can be used to produce material for DNA extraction by harvesting the seeds from the mature fruit.

Simao *et al.,* 2007, had carried out a study of *H. setaceus* seed germination whereby the effect of light and temperature was studied. In his study, it was found that the optimum temperature is between 25˚C and 30˚C. The minimum temperature for germination was between 5˚C to 10˚C whereas the maximum was between 45˚C to 50˚C. It was also reported that the seeds do not germinate in complete darkness. Therefore, the experimental design was adapted from the study with slight modifications. The aim of this chapter is to establish an efficient seed germination method.

3.2 MATERIALS AND METHODS

3.2.1 Plant Materials

Six fully ripened and defect free *Hylocereus* fruits were obtained from a market in Petaling Jaya around 5km from the University (laboratory). Three of the fruits were deployed into experiment immediately (Group 1) whereas the remaining three fruits were stored in the cold room (4^{o}C) for one week before proceeding with the experiment (Group 2). The fruits were cut into half and then the peels were removed revealing the pulps. Seeds from both groups were separated from the flesh using a strainer and subjected to three types of treatment; untreated, acid wash and air-dried. The untreated seeds served as a control in seed germination.

3.2.2 Acid Wash Treatment

Seeds from both groups were treated in a 1L beaker which contained concentrated sulphuric acid (Systerm) (1.25% v/v). The mixture were then stirred using a magnetic

stirrer for 45 minutes, rinsed with running water and dried at 25^{o}C ± 2 °C using a hand towel for 24 hours. The seeds from both groups were then placed in the respective petri dishes and labelled respectively.

3.2.3 Air-dried Treatment

The seeds from both groups were air-dried at 25^{o}C ± 2 °C for 24 hours. The seeds from both groups were then placed in their respective petri dishes and labelled accordingly.

3.2.4 Untreated

The seeds from both groups to be in this treatment were arranged neatly in their respective petri dishes and labelled accordingly.

3.2.5 Germination

One hundred seeds from each treatment (3.2.2, 3.2.3 and 3.2.4) were arranged on two layers of water imbibed filter paper in each of 50mm diameter petri dishes and carried out in triplicates. The petri dishes were wiped with hand towel every 2 days to remove condensed vapour to prevent fungal contamination. Two millilitres of sterile distilled water were added to wet the filter papers to maintain the moisture and observed for seed that successfully germinated. Seeds with at least 1mm long root were considered as germinated (Simao *et al.*, 2007). For each treatment, three petri dishes were placed under fluorescent light (90 lm/W) whereas another three petri dishes were placed in a dark room. This whole process was repeated with fruits initially stored in a cold room for one week. Figure 3.1 and Figure 3.2 shows graphical representation of the germination process whereas Figure 3.3 shows a pictorial representation of seed germination.

Seeds from fruits deployed into experiment immediately

Treatment method

Germination at room temperature with or without light

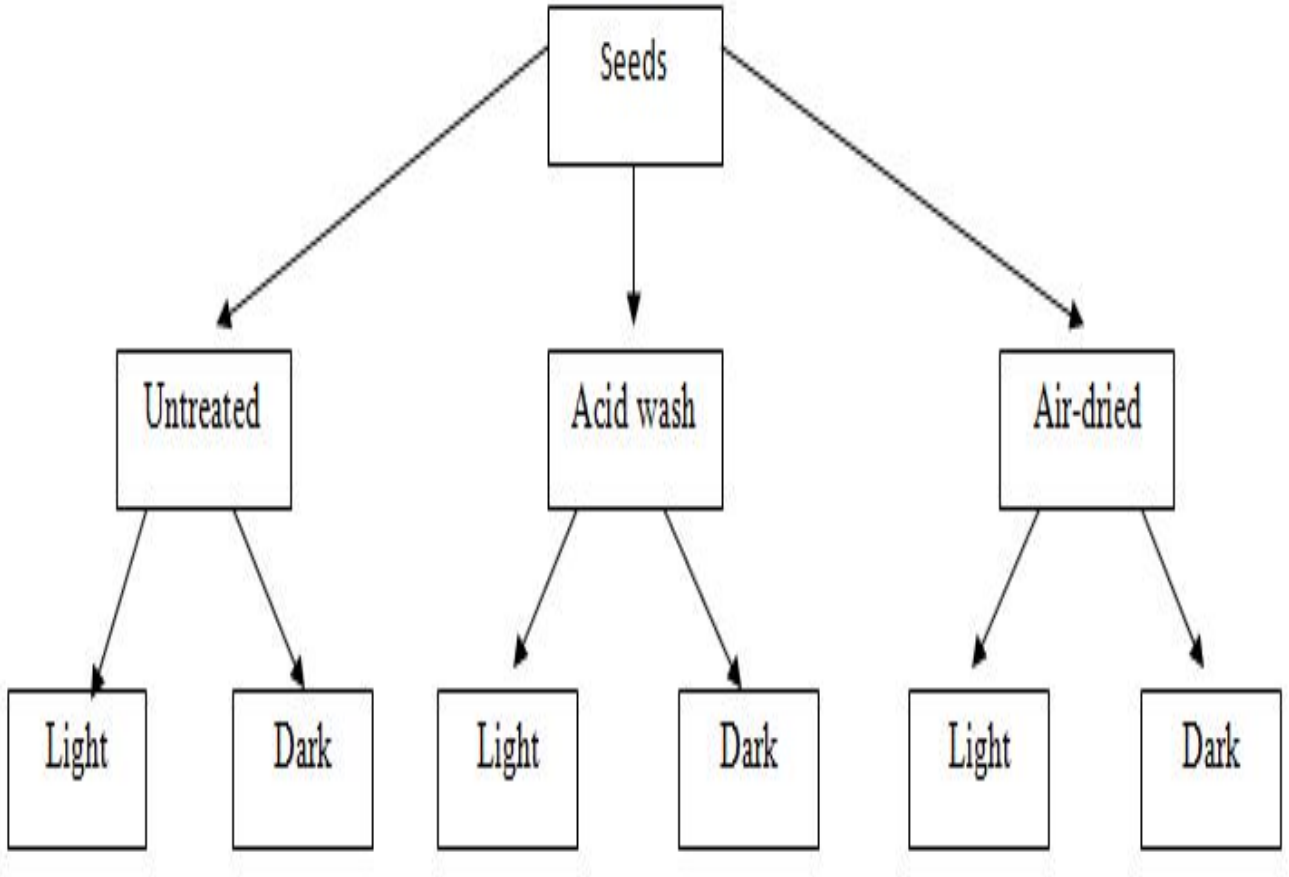


Fig 3.1: Flow chart of seed germination (not subjected to cold storage)

Seeds from fruits stored in cold room for 1 week before being deployed in the experiment

Treatment method

Germination at room temperature with or without light

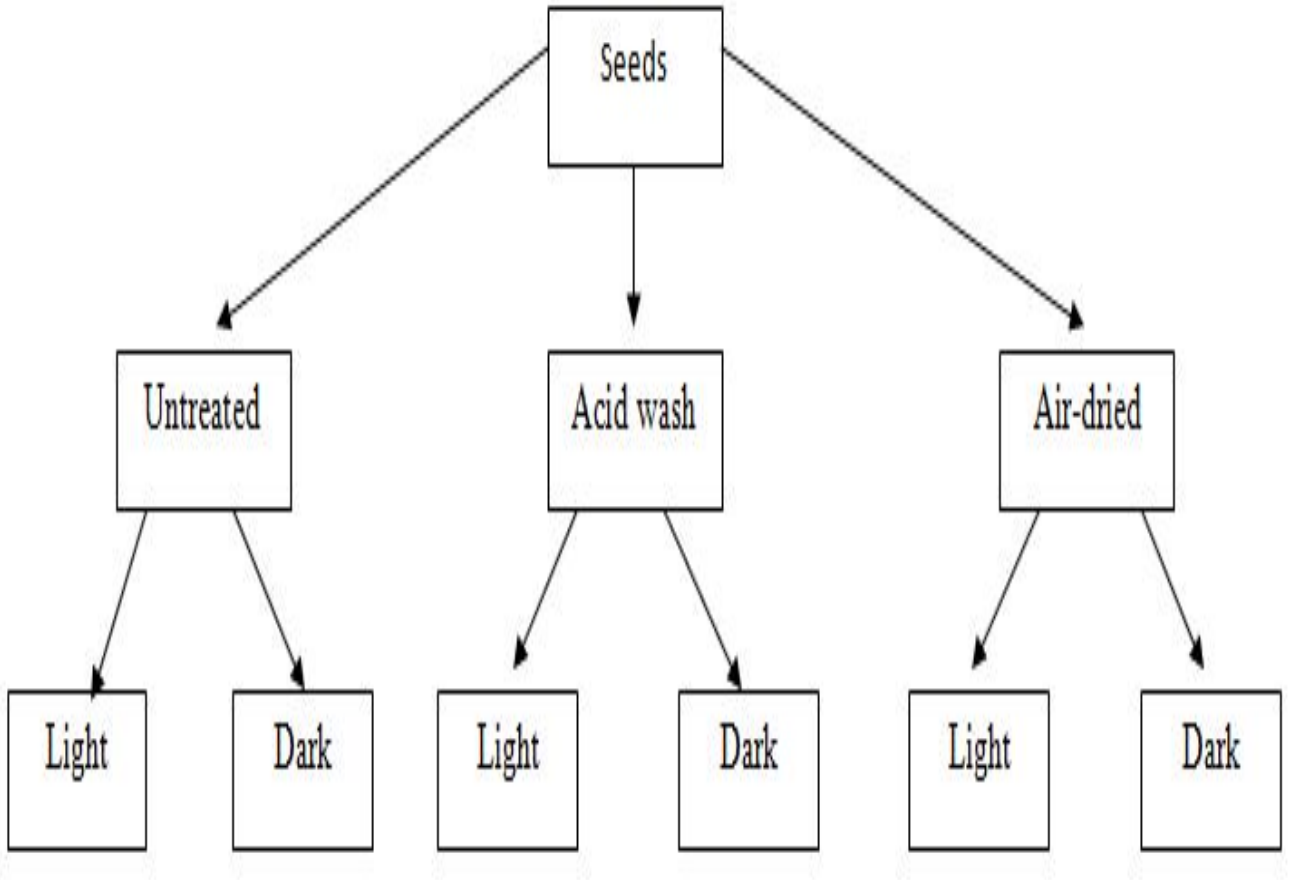


Fig 3.2: Flow chart of seed germination (subjected to cold storage)

a) Hylocereus fruit

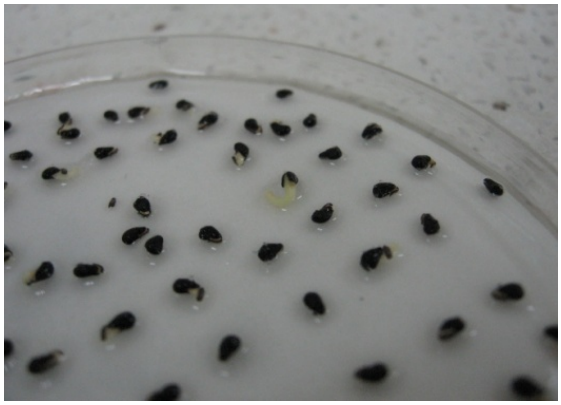
b) Seeds separated from fruit deployed into experiment

c) Seeds separated from fruit subjected to acid wash

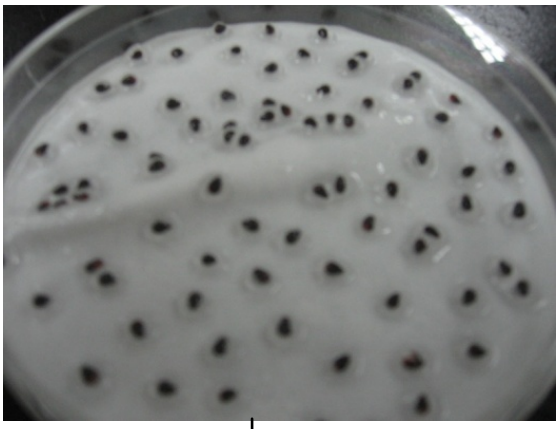
d) Seeds separated from fruit subjected to air dry

e

e) Seeds from (b), (c) and (d) were placed in petri dishes for germination (Day 0)



f) Germination (Day 2)



g) Young leaflets appeared at Day 6

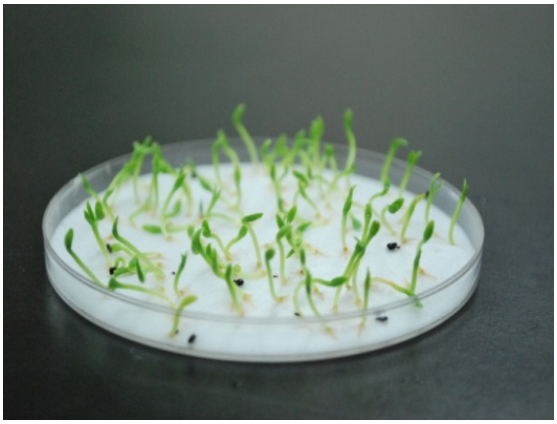


Fig 3.3: Pictorial representation of seed germination. Seeds from both groups were subjected into 3 treatments (untreated, acid wash and air dried). Treated seeds were then placed into petri dishes and allowed to germinate.

3.3 RESULTS

Fresh fruit seeds were subjected to different treatments with the end point being 100% germination.

3.3.1 Germination Percentage of Seeds Harvested from Fresh Fruit under Fluorescent Light and Dark Room

Based on the Figure 3.4, it was found that the seeds exposed to light achieved full germination faster with relative to seeds kept in the dark room. The seeds subjected to light achieved 100% germination at Day 6 whereas the seeds kept in dark room achieved 100% germination at Day 10.

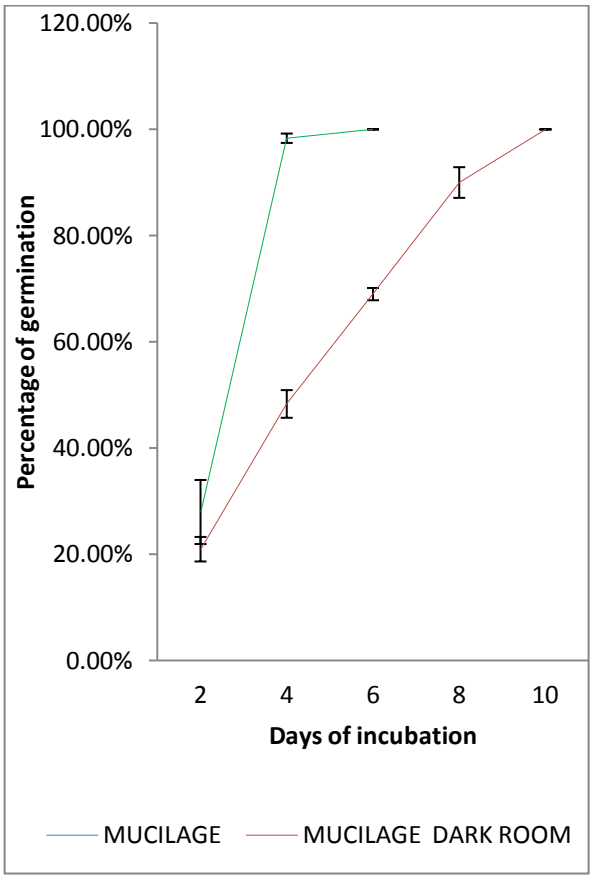


Fig 3.4: Germination percentage of seed harvested from fresh fruit under fluorescent light and dark room. Both group of seeds (exposed to light and kept in the dark room) achieved 100% germination. However, the seeds exposed to light took a relatively shorter period as compared to the seeds kept in the dark room. Vertical bars indicate standard error.

3.3.2 Germination Percentage of Acid Washed Seeds Harvested from Fresh Fruit under Fluorescent Light and Dark Room

Based on the Figure 3.5, it was found that the acid washed seeds exposed to light achieved full germination faster with relative to acid washed seeds kept in the dark room. The acid washed seeds subjected to light achieved 100% germination at Day 6 whereas the acid washed seeds kept in dark room achieved 100% germination at Day 10.

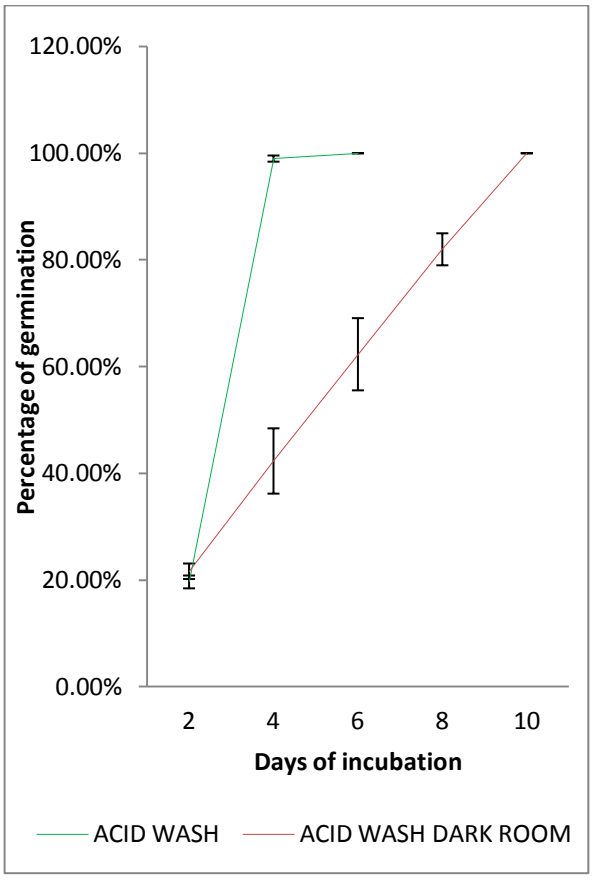


Fig 3.5: Germination percentage of acid washed seeds harvested from fresh fruit under fluorescent light and dark room. Both group of acid washed seeds (exposed to light and kept in the dark room) achieved 100% germination. However, the acid washed seeds exposed to light took a relatively shorter period as compared to the seeds kept in the dark room. Vertical bars indicate standard error.

3.3.3 Germination Percentage of Air Dried Seeds Harvested from Fresh Fruit under Fluorescent Light and Dark Room

Based on the Figure 3.6, it was found that the air dried seeds exposed to light achieved full germination faster with relative to air dried seeds kept in the dark room. The air dried seeds subjected to light achieved 100% germination at Day 6 whereas the air dried seeds kept in dark room achieved 100% germination at Day 10.

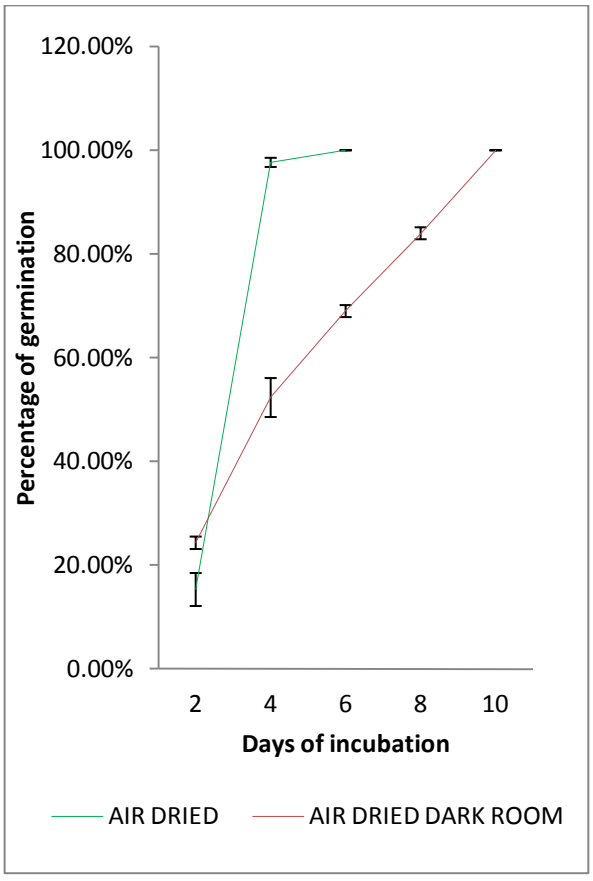


Fig 3.6: Germination percentage of air dried seeds harvested from fresh fruit under fluorescent light and dark room. Both group of air dried seeds (exposed to light and kept in the dark room) achieved 100% germination. However, the air dried seeds exposed to light took a relatively shorter period as compared to the air dried seeds kept in the dark room. Vertical bars indicate standard error.

3.3.4 Germination Percentage of Seeds Harvested from Fruit Stored in Cold Room for One Week under Fluorescent Light and Dark Room

Based on the Figure 3.7, it was found that the seeds from fruits stored in cold room for 1 week prior to being deployed in the experiment and exposed to light achieved full germination at Day 10 whereas seeds kept in the dark room only achieved 21% germination at Day 26.

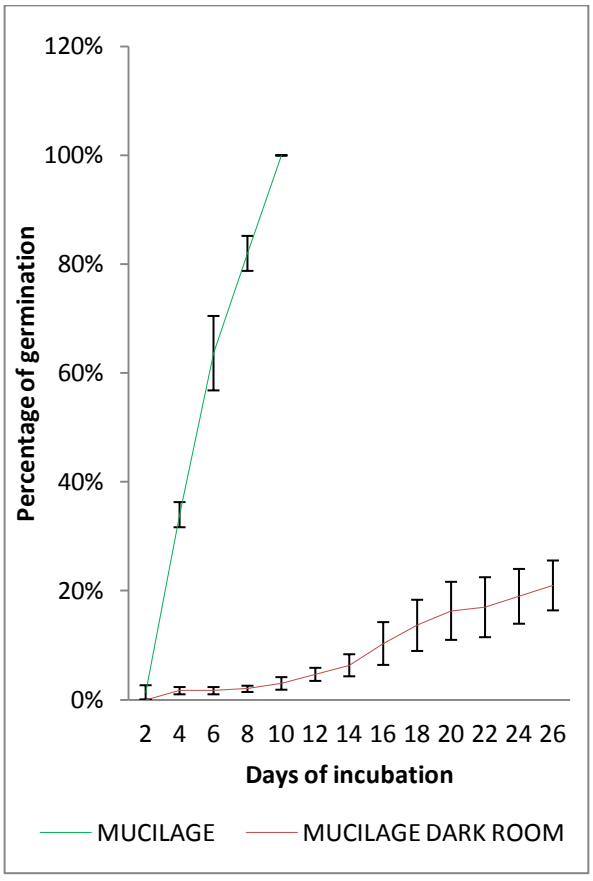


Fig 3.7: Germination percentage of seed harvested from fruit stored in cold room for 1 week under fluorescent light and dark room. Only seeds exposed to light achieved 100% germination. Vertical bars indicate standard error.

3.3.5 Germination Percentage of Acid Washed Seeds Harvested from Fruit Stored In Cold Room for One Week under Fluorescent Light and Dark Room

Based on the Figure 3.8, it was found that the acid washed seeds from fruits stored in cold room for 1 week prior to being deployed in the experiment and exposed to light achieved full germination at Day 10 whereas acid washed seeds kept in the dark room only achieved 43% germination at Day 30.

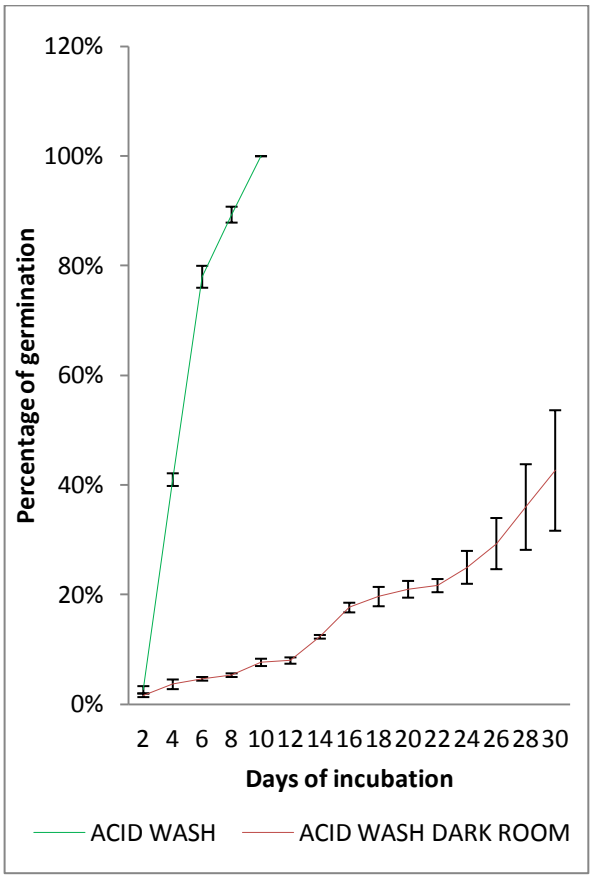


Fig 3.8: Germination percentage of acid washed seeds harvested from fruit stored in cold room for 1 week under fluorescent light and dark room. Only seeds exposed to light achieved 100% germination. Vertical bars indicate standard error.

3.3.6 Germination Percentage of Air Dried Seeds Harvested from Fruit Stored In Cold Room for One Week under Fluorescent Light and Dark Room

Based on the Figure 3.9, it was found that the air dried seeds from fruits stored in cold room for 1 week prior to being deployed in the experiment and exposed to light achieved full germination at Day 10 whereas air dried seeds kept in the dark room only achieved 76% germination at Day 30.

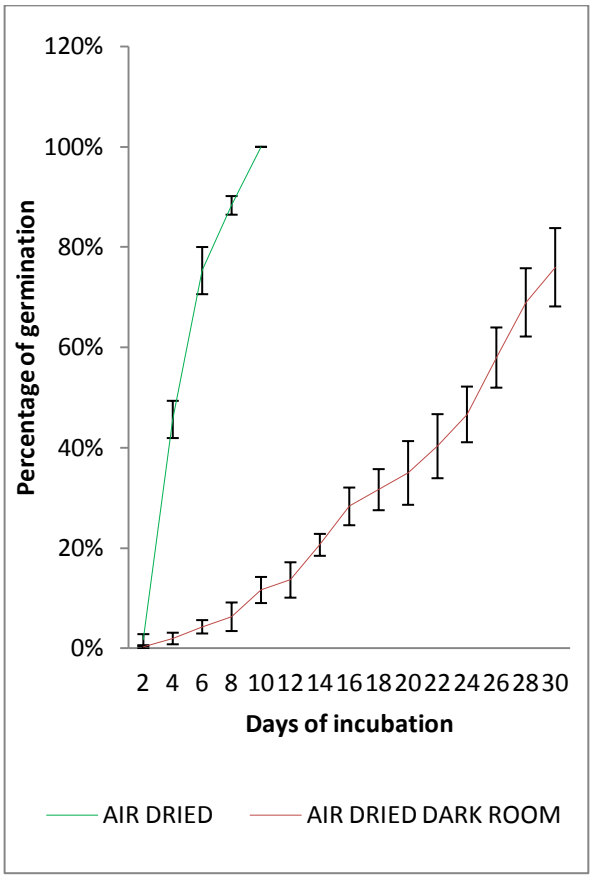


Fig 3.9: Germination percentage of air dried seeds harvested from fruit stored in cold room for 1 week under fluorescent light and dark room. Only seeds exposed to light achieved 100% germination. Vertical bars indicate standard error.

3.3.7 Germination Percentage of Seeds from Fruits Stored In Cold Room Which Were Initially Kept In the Dark Room for One Week And Subsequently Exposed To Fluorescent Light

Based on the Figure 3.10, it was found that seeds from the 3 treatment groups initially kept in the dark room for 1 week showed a relatively low germination rate as compared to when after exposed to fluorescent light. Upon exposure to light, all 3 groups achieved 100% germination. The air dried and acid washed seeds achieved 100% germination at Day 12 whereas the untreated seeds achieved 100% germination at Day 18.

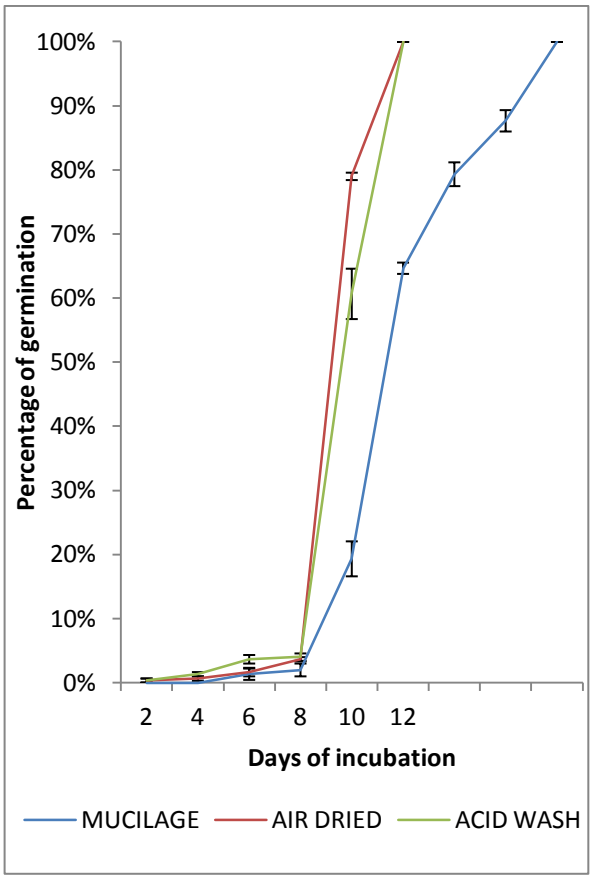


Fig 3.10: Germination percentage of seeds from fruits stored in cold room which were initially kept in the dark room for one week and subsequently exposed to fluorescent light. Seeds kept in the dark room initially showed a relatively low germination rate. Seeds exposed to light achieved 100% germination whereby air dried and acid washed seeds achieved 100% germination at Day 12 and untreated seeds achieved 100% germination at Day 18. Vertical bars indicate standard error.

3.3.8 Germination Percentage of Seeds Harvested From Fresh Fruit under Fluorescent Light

Based on the Figure 3.11, it was found that the untreated, air dried and acid washed seeds harvested from fresh fruit under the fluorescent light achieved 100% germination at Day 6. There were no significant differences between the seeds from the 3 treatment groups in terms of 100% germination being the end point.

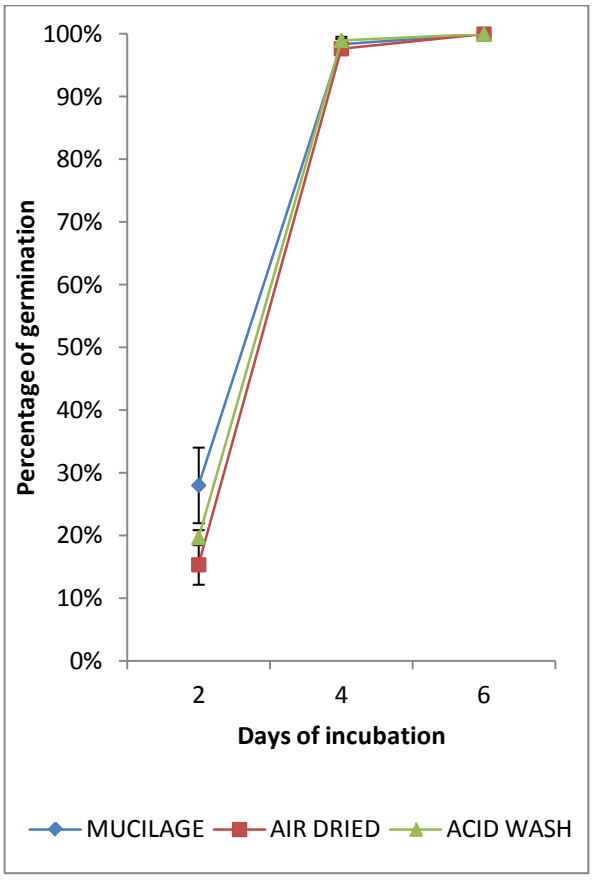


Fig 3.11: Germination percentage of seeds harvested from fresh fruit under fluorescent light. All seeds 3 treatment groups achieved 100% germination at Day 6. There were no significant differences found among the 3 groups. Vertical bars indicate standard error.

3.4 DISCUSSION

Similar study was previously carried out using *Hylocereus setaceus* seeds by Simao *et al.,* 2007. Hence, the experimental design was adapted from the study with slight modifications to suit this current study. An example of the modifications, sulphuric acid was used in this study to remove the pulp from the seeds instead of acetic acid. Seeds were treated with acid to remove the mucilage layer. The maximum germination rate was also stated in Simao *et al., 2007*, which was achieved in a period of one week incubation using seeds from *H. undatus* incubated at 25˚C. In this study, only six days were required to reach a germination rate of 100% at 25˚C under fluorescent light. The germination of cactacean seeds is usually a fast event upon the availability of water. In arid conditions, the seed germination process can be shortened and the time for complete seed imbibitions is also short leading to a high germination rate.

Fluorescent light may play a role in promoting or regulating the germination process. This was because it took a longer duration to reach 100% germination in the dark room. This fact was also supported by evidence which is more obvious when the seeds were kept in a dark room for a week and then exposed to fluorescent light. A sudden increase in germination rate can be observed in the second phase. Nevertheless, light is not a prerequisite factor for germination as the seed can still germinate but only at a much slower rate. Simao *et al.,* 2007 suggested that phytochrome could control seed germination in *H. setaceus.* According to Pearson *et al.,* 2003 small seeds usually respond positively to the light influences, while large seeds usually respond positively to temperature fluctuations.

In this study, it was found that seeds obtained from fruits initially stored at cold temperature could also undergo normal germination process. However, the time taken to achieve complete germination is relatively longer as compared to those seeds obtained from fruits not stored at cold temperature. The seeds obtained from fruits initially stored at cold temperature required an extra of 4 days to reach 100% germination. This may be due to the fact that the germination process is influenced by the enzyme activity regulated by temperature or low temperature storage for a week that induce dormancy.

In the germination process, the seed starts with rapid uptake of water that causes the seed coat to swell and eventually lead to radical emergence. The exact mechanism for the radical protrusion is still unknown and may due to the osmotic potential of the radicle cells becomes more negative which would lead to increased water uptake and cell extension, expansion of radical cell wall and breakdown of the seed tissues surrounding the radical tips (Mei and Song, 2008). Koornneef *et al.*, 2002 reported that tomato and tobacco germination was controlled by interactions between the embryonic radicle tip and the enclosing endosperm cap. Enzymatic hydrolysis weakens the endosperm cap which led to radical protrusion. Enzymes responsible in this process were expansin and endo-β-mannanase, which are specifically expressed in the endosperm cap of tomato.

Enzymes are hydrated during the rapid uptake of water subsequently which led to hydrolysis of the storage reserved to provide energy for cell expansion and synthesis of plumul. In the research by Mei and Song, 2008 contents of solubles sugar and starch gradually decreased, and the activities of α-, and β-amylase increased with imbibition, the decrease in soluble starch content was due to hydrolysis by increasing α-, and β-amylase

activities. As the cotyledons become exhausted, the seedling is faced with the need to maximise the production of aboveground biomass in order to achieve an optimal resourceforaging balance with the resulting of thin nitrogen-rich leaves with a high photosynthetic capacity (Hanley *et al.*, 2007).

The seed coat of an inmature fruit are light brown colour, black when is mature. The colour change was due to the oxidation of the flavoniod compounds by laccase-type polyphenol oxidase. Due to the antimicrobial properties of flavonoids, it may serve as chemical barrier which prevents fungus invasion (Rajjou and Debeaujon, 2008). However, long storage period may lead to breakdown of the flavonoids which renders the seeds susceptible to fungus invasion and the surrounding mucilage may promote fungus growth. Hence part of the experiments terminated early due to fungal contamination. In this study, the seed germination was limited to 30 days due to fungal contamination.

Seeds of *H. undatus* fruits not exposed to cold environment for one week have the best germination percentage when placed in continuous fluorescent light at room temperature. This method is able to provide a readily available source of raw material for DNA extraction. There was no dormancy observed in this study as all seeds exposed to different environments achieved 100% seed germination. Although all three groups of seeds will ultimately reach the maximum endpoint of 100% seed germination, it is best to use seed either acid washed or air dried to minimise fungal contamination.

In a nutshell, all seeds managed to germinate although requiring different period of time influenced by environmental factors. Seed germination is influenced by the availability of water, light and temperature. Duration of seed germination can be optimized by manipulating the variables stated above. Presence of water and light as well as temperature at 25˚C shortens the time taken for a seed to germinate.