agronomy

MDPI

*Article*

# Seed Quality of Lablab Beans (*Lablab purpureus* L.) as Influenced by Drying Methods and Storage Temperature

**Aliyah Mohd Yahaya, Uma Rani Sinniah * and Azizah Misran**

Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, Seri Kembangan 43400, Selangor, Malaysia; aliyahmohdyahaya@gmail.com (A.M.Y.); azizahm@upm.edu.my (A.M.)

* Correspondence: umarani@upm.edu.my; Tel.: +60-172-551-500 or +60-397-694-839

**Abstract:** Drying and storage are the common postharvest issues in seed production. Normally, seeds are harvested at physiological maturity when the moisture is higher than desired for safe storage. This study aims to evaluate the use of common drying methods and suggest a suitable storage temperature for the lablab bean seed. Pods at 30 days after anthesis are harvested and the seeds subjected to drying using sun, shade, oven, and drying beads to obtain target moisture contents of 14%, 12%, and 10%. Dried seeds are then stored at two storage temperatures; ambient (27.7–34.2 °C; 74 ± 5% relative humidity) and refrigerated (2.0–5.0 °C; 25 ± 5% relative humidity) for six months. Both shade and drying beads provided good results. However, drying beads gave the highest germination percentage (≥70%) and the shortest drying duration to achieve safe moisture content of 10%, along with the economic benefit from the repeated usage. The beads can be reused indefinitely by recharging in an oven between uses, thus, indicating the potential adoption by farmers. Two commonly used methods (sun and oven) have been proven to be unsuitable as they increase dead seeds due to heating damage. It is further explained that during six months of storage in the ambient temperature, declines were recorded in antioxidant enzyme activities, germination performance, and seedling growth. Therefore, lablab bean seeds are best stored refrigerated (≤5 °C) in which the germination and defense mechanism are maintained, and it is highly recommended to the farmers and seed producers as it is easily accessible, cost-saving, and sustainable.

**Keywords:** lablab; seed drying; moisture content; germination; storage temperature; antioxidant enzyme



**Citation:** Yahaya, A.M.; Sinniah, U.R.; Misran, A. Seed Quality of Lablab Beans (*Lablab purpureus* L.) as Influenced by Drying Methods and Storage Temperature. *Agronomy* **2022**, *12*, 699. https://doi.org/10.3390/agronomy12030699

Academic Editor: Cristina Patanè

Received: 18 January 2022
Accepted: 4 March 2022
Published: 14 March 2022



## 1. Introduction

*Lablab purpureus* is a high-value legume that has the potential for being an important vegetable due to the high protein content (25%). Recently, a purple variety has been popularized as it has natural antioxidants that have numerous medicinal advantages, such as anti-cancer and anti-diabetic properties [1]. With such potentials, the bean has gained significant importance in the national food supply chain. However, one of the major constraints in producing lablab bean would be the insufficient supply of quality seeds.

Seed quality is dependent on seed handling and postharvest practices. Controlling the various postharvest factors, such as drying and storage, where environmental factors such as moisture, temperature, and relative humidity play important roles in producing high quality seeds. There is a report on large postharvest losses in lablab due to the use of sun drying method. Currently, the method is used where lablab bean seeds are left to dry on pavement before being stored and, resulting in a loss of 5–10% seeds worth millions of dollars [2]. By applying the sun-drying method, seeds are exposed to the natural environment, which is beyond human control. The removal of moisture from the seed depends solely on the relative humidity and temperature of the natural environment, which is proven to reduce the germination and vigor, a threat to lablab seed quality. Therefore, different kinds of drying approaches are needed for the evaluation of lablab bean seeds.

The purple lablab bean is found best to harvest at physiological maturity, which is 30 days after anthesis (DAA), as mentioned by Das and Fakir [3]. Despite having maximum seed quality in terms of germination and vigor at physiological maturity, Refs. [4,5] drying is a necessary step, as according to Lassim and Chin [6], seeds collected at this stage usually have high moisture content. It is reported by Bewley [7] that seeds are susceptible to aging when the moisture content at harvest is high ($\geq$14) and must be dried quickly to prevent further deterioration and to preserve maximum quality and longevity. The above-mentioned environmental conditions during the process of seed drying may lead to changes in chemical composition of the seed and influence the final seed quality. The term "seed quality" is used in agriculture to describe the overall value of a seed lot, which encompasses germinability, vigor, genetic and physical purity, and seed health. Consequently, environmental conditions, such as temperature and relative humidity, influence the quality of seeds throughout the postharvest periods.

There are many common and economical ways for drying seeds, such as drying under the sun and shade. A more sophisticated method is used for high-value seeds, such as refrigeration drying with low relative humidity, but it is concluded that response to drying can be species specific which depends on the ability to tolerate desiccation. In addition, it is reported by Berjak and Pammenter [8], that a wide gradient of desiccation sensitivity or tolerance exists among legumes. For example, green peas are highly sensitive towards oven drying as germination drops when the oven method is applied. As for a study conducted by Krzyzanowski et al. [9], it is proven that the effects of drying in an air-conditioned room at low humidity is best for the seed quality of peanut (*Arachis hypogaea*, L.). In his study, seed moisture dropped from 17.4% to 7.3%, and there was an improvement in seed germination. The above examples clearly imply that the drying process directly affects seed performance. However, publications on lablab bean seed production are limited, and thus, none are available for the effect of drying. Hence, it is important to acknowledge this gap by applying a suitable economical postharvest drying method for lablab bean seeds to maintain the seed quality.

Storage temperature is also an important factor that determines seed quality. According to Justice and Louis [10], during the time that seed is held in storage, there will be a gradual decline in germination and vigor. It is reported that farmers currently stored lablab bean seeds under ambient conditions, as it is the most common practice while considering the point of view of economic affordability. However, various literatures have addressed the bad effects of storing seeds under ambient conditions as there are several factors that influence ambient temperature, including the humidity, weather, quality of insulation in the room, and usage of any heating or cooling systems.

Panda [11] has stated that the viability of many seeds is reduced by half within six months under ambient storage conditions. This is in line with a study by Nagaveni [12] who finds that seeds stored under ambient conditions (16–30 °C and 30% RH) deteriorate at a faster rate and have lower germination and vigor as compared to those seeds stored under closed conditions for 27 months. Henceforth, it is very crucial for seed moisture and storage temperature to be kept low to improve storability. Unfavourable ambient factors (high humidity and temperature) can accelerate seed deterioration and trigger microbial and fungal activity. It is further reported that the deterioration is caused by reactive oxygen species (ROS), usually produced from stress conditions, such as high temperature during storage. The ROS accumulation is a significant contributor to seed degradation, and enzyme antioxidant systems play a critical role in scavenging ROS during seed ageing. According to Jyoti and Malik [13], the accumulation can be destroyed by the activity of scavenger enzymes, such as catalase and peroxidase. This scavenger enzyme needs to be preserved to maintain seed quality.

With the current practices, the lablab bean seed quality may have been compromised. Therefore, it is important to address the issues regarding the postharvest handling of lablab bean seeds, particularly during drying and storage. This will be helpful to promote

the potential of the lablab bean in food security and further expand the seed production program in Malaysia.

## 2. Materials and Methods

### *2.1. Study Location*

A total of sixty plants were grown for seed collection. The study was carried out during the period of February 2018 to August 2019 at the Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia (UPM), Serdang, Malaysia. The lablab cultivar, MDI12839 (purple-coloured variety), was used in this experiment. The reproductive growth stages and development of the lablab bean were recorded based on the method described by Singh and Abhilash [14]. Since the seed maturity stages are very important, flowers were tagged with coloured threads at the first opening of flowers to collect 100 pods on the same day. Pods were then harvested weekly after they changed from purple to a yellowish-brown colour, which happened around 30 days after anthesis (DAA), an indicator of physiological maturity (PM).

### *2.2. Seed-Drying Treatments*

Prior to the drying treatments, initial seed moisture content was determined using the high constant temperature oven method suggested by ISTA [15]. Then, the collected pods of lablab bean were dried to the different target moisture contents of 14%, 12%, and 10% using different methods of drying, such as sun drying, shade drying, oven drying at 35 ± 2 °C, and using drying beads. The process of each drying treatments was explained in Table 1.

**Table 1.** Seed drying treatments and processes for lablab bean seeds used in this study.

| Seed Drying Treatments | Seed Drying Process |
|---|---|
| Sun | Lablab bean seeds were sundried by spreading a thin layer of pods on drying mat at temperatures of 29–37 °C; 74% RH and left under the sun until TMC was achieved. Seeds were sun-dried for 5 h/day and kept in the laboratory at night and further dried on the next day. |
| Shade | Lablab bean seeds were placed under 50% shade by spreading a thin layer on drying mat at temperature of 27 °C; 74% RH until TMC was achieved. Seeds were shade dried for 5 h/day and kept in the laboratory at night and further dried the next day. |
| Oven | Lablab bean seeds were dried under low temperature oven drying, 39 °C, until TMC was achieved. |
| Drying beads | Lablab bean seeds were dried using drying beads at 1:1 bead to seed ratio in airtight plastic containers. Seeds were placed in a container with the beads and left to dry. |

The target moisture content (TMC) was further determined by using the oven method, and seeds were weighed from time to time, and drying was stopped when TMC was achieved, and the drying duration was recorded. The determination of target moisture content was done using prediction of final seed weight as the indicator for the accurate drying period and was calculated as below:

$$\text{Final Seed Weight} = \text{Initial Seed Weight} \times \frac{(100 - \text{Initial Moisture Content})}{(100 - \text{Final Moisture Content})} \quad (1)$$

Once the seeds reached the TMC, seed quality assessments were further performed with viability determined by calculating the germination percentage based on ISTA [15], while vigour was calculated by the seed vigour index (SVI) and the germination rate

index (GRI) according to Abdul Baki and Anderson [16] and Kader [17], respectively. All treatments were arranged in a factorial experiment based on randomized complete design (CRD) with three replications.

#### 2.2.1. Seed Moisture

Seed moisture content (fresh weight basis) was determined using three replicates of ten seeds from each treatment and were subjected to oven drying using the high constant temperature method (130 °C for 1 h), by ISTA [15]. The moisture content was calculated using the following formula:

$$\text{Moisture content } (\%) = \frac{W2 - W3}{W2 - W1} \times 100 \quad (2)$$

where, $W1$ = weight of the container with lid, $W2$ = weight of the container with lid and sample before drying, and $W3$ = weight of the container with lid and sample after drying.

#### 2.2.2. Germination and Vigour

Once the seeds reached the targeted moisture content, viability was determined by calculating germination percentage. The germination test was conducted with three replications for each experimental unit at room temperature (28 ± 2 °C) using sterilized sand as described by ISTA [15]. Seed germination was counted daily for 14 consecutive days. On the final day, the number of normal seedlings was counted and expressed as germination percentage by using the following formula:

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 10 \quad (3)$$

Seedling vigour index (SVI) was calculated as described by Abdul-Baki and Anderson [16]. As for the germination speed, the daily germination count data was used to calculate the germination rate index (GRI) to determine the speed of germination [17].

#### 2.2.3. Electrical Conductivity of Leachates

Electrical conductivity is a test to measure viability and seedling vigour [18]. A total of 20 seeds per replicate were used for each treatment and placed in a vial containing 80 mL distilled water. Then, they were placed at 25 °C for 24 h. After 24 h of soaking, seeds were then swirled for 10–15 s, and the conductivity meter was dipped into the cell in the water until a stabilized reading was achieved and recorded. The mean of three plastic vials containing distilled water only (control) was measured and served as the background reading. The electrical conductivity test was calculated as per the formula below by ISTA [15].

$$\text{Conductivity test } (\mu S \cdot cm^{-1} \cdot g^{-1}) = \left(\frac{\text{Conductivity reading } (\mu S\ cm^{-1}) - \text{background reading}}{\text{weight of replicate } (g)}\right) \quad (4)$$

The leakage on membrane permeability was further determined by using dye staining to monitor the leakage. Two replicates of ten seeds were immersed in 0.1% Safranin red-dye-staining agent or control (distilled water). The seeds were then dissected and the staining pattern was observed under a stereomicroscope (Leica EZ4D) 17 h after the imbibition is started.

### *2.3. Post-Drying Storage Temperature*

The experiment was further followed by storing the dried lablab bean seeds of 14%, 12%, and 10% MC under two storage temperatures, ambient (27.7–34.2 °C; 74 ± 5% RH) and refrigerator (2.0–5.0 °C; 25 ± 5% RH), using air tight aluminium packaging for six months. Seed quality and seedling performance of stored seeds parameters were measured after six months of storage. Seed quality parameters, such as standard germination, coefficient

velocity of germination, and seedling vigour index, were measured based on three replications according to the methods previously described. During the storage period, the changes in the biochemical process involved in seed deterioration under different storage temperatures were also monitored. During storage, it was important to detect the activity of defensive mechanisms (catalase (CAT) and peroxidase (POD) activities) available as it reflected on the lablab bean seed quality.

Antioxidant Enzymes

The activity of the catalase and peroxidase enzymes were detected using a spectrophotometer (Shimadzu UV-3150 UV-VIS Near IR, Shimadzu Corporation, Kyoto, Japan). Ground lablab bean powder (0.15 g) was homogenized with 1.5 mL of 100 mM potassium phosphate buffer (pH 7) using a cold mortar and pestle. Each extracted sample was centrifuged at 13,500 rpm for 20 min at 4 °C. The supernatant was used to determine the CAT and POD activities. Three replications were tested for each treatment combination. Catalase (CAT) activity was calculated according to the method described by Aebi [19], and the activity unit was presented as per milligram of extractable fresh weight (μmol/min/mg/FW). The guaiacol peroxidase (POD) activity was measured as described by Maehly [20]. The POD enzyme activity was expressed as per milligram of extractable fresh tissue.

### *2.4. Data Analysis*

Analysis of variance (ANOVA) of the data appropriate to the experimental design and comparison of means were compared by least significance difference (LSD) at $p < 0.05$. All statistical analyses were carried out using Statistical Analysis System version 9.4 (SAS Institute Inc., Cary, NC, USA).

## 3. Results

### *3.1. Seed and Seedling Quality Based on Different Drying Methods*

Seed drying is an important process prior to storage where physiological (moisture content and dry weight) and functional (vigour and viability) changes need to be maintained to ensure seed respiration continues at low levels to keep embryos alive. Results have revealed that different methods of drying influence the time taken to achieve the target moisture content (14%, 12%, and 10%). The duration of each drying methods to achieve the targeted moisture content is presented in Figure 1, and the influence of drying methods on the seed germination is further explained below.

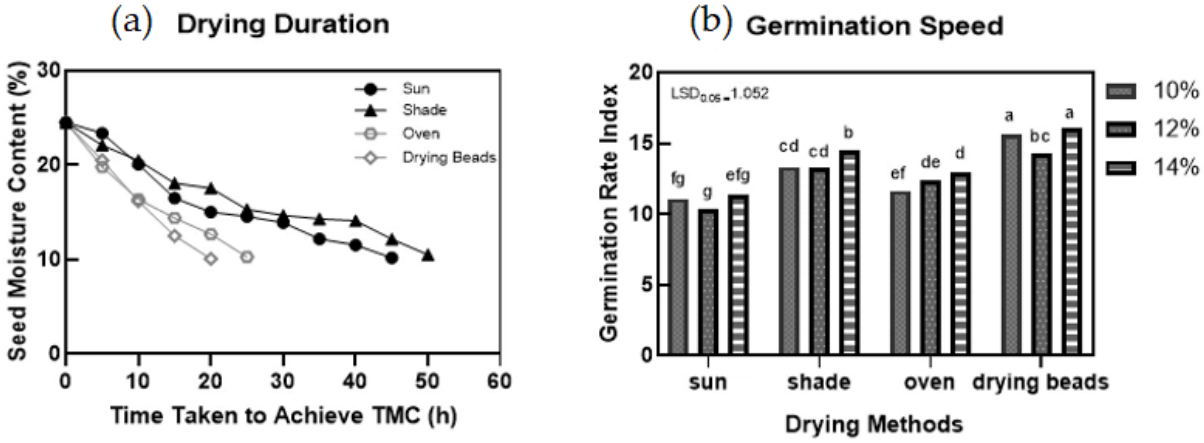


**Figure 1.** Effects of different drying methods (sun, shade, oven, and drying beads) on drying duration (**a**) and germination speed (**b**) of lablab bean seed. Means with same letters are not significantly different.

Figure 1 shows that lablab bean seeds dried using the oven and drying beads attained target moisture content within 25 h, while those dried under the sun and shade required up

to 50 h to reach 14%, 12%, and 10% MC. Based on the recorded times, the drying methods can be divided into two groups which are slow drying (sun and shade) and rapid drying (drying beads and oven).

I. Rapid Drying

Removal of moisture is found to be very fast with the rapid drying technique. Both drying beads and the oven achieved 14% MC within 10 h and 12% achieved at 15 h, respectively. However, differences occur when drying is extended to achieve 10% MC. The shortest time taken is by using drying beads; it took 20 h to achieve 10% MC compared to oven drying at 25 h. The rapid rate of drying using beads agrees with the finding of Nassari et al. [21], whereby the drying in tomato seeds is faster compared to sun-drying. They dried seeds rapidly due to the strong affinity to absorb and hold water very tightly in their microscopic pores as reported by Atilia and Zaitialia [22]. This is also the safest method when leaving the seeds in an environment of low relative humidity and allowing the seed moisture content to reach equilibrium. It is significant to understand that the process of moisture removal from the seed is greatly dependent on the relative humidity and temperature of the natural environment. Hence, with drying beads, the seeds are able to dry rapidly within a day under varied situations compared to other drying methods.

II. Slow Drying

In contrast, drying under direct sunlight and under shade have similar drying patterns. Both drying methods recorded longer times to achieve the lowest targeted moisture content of 10% which requires up to 50 h. The removal of moisture is found to be slow as the water moves freely at the surface of seeds, and the moisture removal is greatly dependent on weather conditions. A study by Ali et al. [23] suggested that soybean seeds should be sun dried to $\leq$8% MC prior to storage. However, according to Hor [24], sun drying caused considerable seed deterioration to groundnuts and are best dried using the oven. Although sun drying is a cost-saving method, commonly used by farmers and seed producers, and is the current practice used by farmers to dry lablab bean seeds, it is reported to be slow and unsuitable for tropical weather conditions, such as in Malaysia, according to Muckle and Stirling [25]. Moreover, sun drying is time and labour intensive and adversely affects seed quality traits, such as germination and vigour.

The summary of seedling performance based on germination and the seedling vigor index are further presented in Table 2.

**Table 2.** Lablab bean seed germination percentages after drying using different methods.

| Source of Variation | Germination (%) | Seedling Vigour Index |
|---|---|---|
| Drying methods | ** | ** |
| Sun | 43.89 c | 12.95 c |
| Shade | 67.78 a | 18.08 b |
| Oven | 58.89 b | 12.24 c |
| Drying beads | 70.56 a | 20.46 a |
| Seed moisture content (%) | ** | ** |
| 10 | 70.83 a | 17.76 a |
| 12 | 58.75 b | 15.84 b |
| 14 | 51.25 c | 14.20 b |
| Drying methods × Seed Moisture Content | ns | ns |

Note: mean values marked with different small letters indicate significant differences at the $p < 0.05$ level. Means with same letters are not significantly different ($p < 0.05$, LSD test); ** = significant at 1% level of probability; ns = not significant. The initial germination of lablab bean seeds was tested at = 63.3%.

According to the germination data in Table 2, different germination results are observed with each drying method. The seed quality analysis revealed that seeds dried using drying beads and shade recorded increases in germination ($\geq$70%), with the highest vigour and germination speed obtained when drying beads are used in comparison with those

seeds dried with the oven, sun, and shade. Drying beads achieved a higher germination percentage (70.56%), followed by shade drying (67.78%), oven drying (58.89%), and sun-dried seeds (43.89%); this is due to low relative humidity (RH) inside the closed container used to dry seeds together with the beads. Using a 1:1 ratio (drying beads: seed) gave the highest drying rate, compared with shade and other methods. This is consistent with other seed drying studies carried out using desiccant drying in which seeds rapidly dried while germination was maintained.

In contrast, the two most common methods, sun and oven drying, significantly increased the number of dead seeds. In addition, this study showed that poor germination ($\leq$59%) and seedling vigour were obtained with both methods. The present study has suggested that lablab bean seeds are sensitive to heat during the desiccation process; thus, oven and sun drying are found to be not reliable for drying lablab bean seeds. This may be due to the heating damage in which the highest temperature recorded under the sun was 45 °C, and according to Nautiyal [26], drying $\geq$ 40 °C will reduce the viability of seeds rapidly. This is supported by Figure 2 showing the comparison on the membrane integrity being greatly reduced when the lablab bean is dried using oven.


**Figure 2.** Comparison of the seed membrane integrity of freshly harvested seeds with seeds subjected to two different drying methods (with drying beads, membrane integrity was less reduced, and with oven drying, membrane integrity was greatly reduced).

These findings are consistent with those observed by Afonso and Correa [27], who have also reported higher drying air temperatures resulted in increased electrical conductivity of bean seeds. Furthermore, the authors found that seeds harvested with high moisture content were more susceptible to loss of quality during drying. It can be concluded that drying seeds using drying beads (under 10–15 RH%) to be the best method that enable seed producers to dry seeds to a low moisture content without the use of heat, thus preserving seed quality. Therefore, the present study shows that drying beads maintain lablab bean seed quality. In addition, the beneficial parts of using drying beads would be the beads can be used repeatedly and is a good investment for seed producers.

During the drying period, an interesting event is found in which lablab bean seeds harvested at 30 DAA only had 63.3% germination and drying improved this to 70.6%, which is still below the accepted 80% germination for commercial seed production. This can be due to seeds being harvested at a stage where they are still underdeveloped (Figure 3). It shows in Figure 3 that the colour changes of the purple lablab occur from 30 DAA, as suggested by Fatin et al. [28], right through 40 DAA. There is a possibility that the purple lablab bean can be harvested later than 30 DAA, and to ensure that maximum viability and vigour are achieved, harvesting at later stages (35–40 DAA) should be practiced for commercial seed production. Moreover, the recommended methods (drying beads and shade drying) are still applicable to farmers for commercial seed production and with the right harvesting maturity, the percentage of germination can be increased.


**Figure 3.** Pod formation and maturation of *Lablab purpureus* L., and changes in pod colour occur from purple to light brown starting at 30–40 DAA.

After lablab bean seeds have been dried, seeds are then stored under two temperatures, which are ambient (27.7–34.2 °C; 74 ± 5% RH) and refrigerator (2.0–5.0 °C; 25 ± 5% RH) for six months. It is important to acknowledge which temperature slows down the ageing process as the seed deterioration process is unavoidable, regardless of storage environments and crop species (Mohammadi et al. [29]; Dona et al. [30]). Based on Table 3, results show different responses occur when seeds are subjected to the two different storage temperatures.

**Table 3.** Lablab bean seedling germination, vigour, and germination speed after six months of storage under two different storage temperatures.

| Source of Variation | Germination (%) | Seedling Vigour Index | Coefficient Velocity of Germination |
|---|---|---|---|
| Storage temperature | ** | ** | * |
| Ambient | 50.67 b | 8.67 b | 16.26 b |
| Refrigerator | 68.89 a | 17.49 a | 21.01 a |
| Seed moisture content (%) | ** | ** | ns |
| 10 | 62.67 a | 14.92 a | 19.593 a |
| 12 | 62.66 a | 13.46 a | 18.57 a |
| 14 | 54 b | 10.87 b | 17.75 a |
| Storage temperature × Seed moisture content | ns | ns | ns |

Note: mean values marked with different small letters indicate significant differences at the $p < 0.05$ level. Means with same letters are not significantly different ($p < 0.05$, LSD Test); * = significant at 5% level of probability; ** = significant at 1% level of probability; ns = not significant. The initial germination of lablab bean seed was tested at = 84.5%.

Seeds stored in the refrigerator (2.0–5.0 °C; 25 ± 5% RH) have maintained higher germination (≥68%) and vigour together with maintained germination speed compared to the ambient temperature (27.7–34.2 °C; 74 ± 5% RH), which drops to ≤50% germination with low vigour after six months of storage. This agrees with Prandhan and Badola [31] that storing seeds under ambient conditions often result in low seed germination and seeds deteriorate faster. It is seen in Figure 4 that the physical quality of lablab bean seeds is better and shiny under the refrigerator temperature compared to the ambient as the seeds become dull and start to rot six months after storage.

A similar pattern is observed for seed moisture content. It was noticed that the seeds initially stored with a 10% moisture content maintained higher germination, vigour, and germination speed after six months of storage. The germination percentage is more or less similar for seeds initially stored with 12% and 14% MC with a range of 55–62% germination.


**Figure 4.** Effects of different storage temperatures and seed moisture content on seed physical conditions of lablab bean seeds.

On the other hand, seed vigour continuously decreased with increasing temperatures and seed moisture content. Lablab bean seeds stored under the refrigerator temperature kept better seedling performance after six months storage. It is found that a higher seedling vigour index was recorded when seeds were initially stored with 10% MC in refrigerated temperature. This is in agreement with Krishna and Pallavi [32] that seeds stored at cold temperatures recorded high seedling vigour after 30 days of storage compared to the ambient storage. A reduction in seedling vigour after 90 days of storage is further observed under ambient temperature over the cold storage. This proved that seeds are best to be stored under refrigerated temperatures, in line with the findings of this study that showed higher seedling vigour throughout six months of storage. Seeds with higher MC (14%) showed loss rapidity and took the longest time to germinate. The reduction in the speed of germination is the visible symptom of reduced vigour. It is noticed that faster seed emergence leading to better germination due to high vigour is found in seeds stored under refrigerated temperatures with initial moisture content of 10%.

### *3.2. Antioxidant Enzyme*

To prolong seed quality and longevity during storage, it is important to reduce the accumulation of ROS, which can be accomplished by ROS-scavenging enzymes in the seeds. Antioxidant enzymes that scavenge ROS consist of catalase (CAT), peroxidase (POD), and dismutase superoxide (SOD), as mentioned by Pukacka and Ratajczak [33].

From the findings, CAT and POD activities are significantly affected by storage temperature and moisture content, as shown in Table 4. The findings indicate a close association between seed quality reduction with enzymes CAT and POD. CAT activities are relatively higher (1.16 µmol/min/mg FW) at six months of storage under the refrigerator temperature, regardless of the seed moisture content. For seeds stored at the ambient temperature, the rate of decline is greater. It is visible from Table 4 that the levels of CAT activities are considerably lower at the ambient temperature which is below 1.0 µmol/min/mg FW. A similar pattern is reported for POD activities. During the six months of storage, seeds stored under refrigerator, regardless of the initial seed moisture content, showed higher readings of POD, and the lowest POD activities are found when seeds are initially stored with 14% and kept under ambient temperature. The results in this study confirmed that reduced CAT and POD activities in ambient temperature are the major cause of seed deterioration during storage at 27.7–34.2 °C; 74 ± 5% RH.

**Table 4.** POD and CAT activity of lablab bean seed after six months of storage under different storage temperatures.

| Storage Temperature | Seed Moisture Content (%) | CAT Activity (µmol/min/mg FW) | POD Activity (µmol/min/mg FW) |
|---|---|---|---|
| Ambient | 10 | 0.38 c | 0.095 c |
| | 12 | 0.38 c | 0.097 c |
| | 14 | 0.35 c | 0.091 c |
| Refrigerator | 10 | 1.76 a | 0.26 b |
| | 12 | 1.23 b | 0.31 b |
| | 14 | 1.16 b | 0.50 a |
| Storage temperature × Seed moisture content | | ** | ** |
| LSD 0.05 | | 0.11 | 0.12 |

Note: mean values marked with different small letters indicate significant differences at the $p < 0.05$ level. Means with same letters are not significantly different ($p < 0.05$, LSD Test); ** = significant at 1% level of probability.

This indicates that the defensive mechanism of seeds stored under the refrigerated temperature is maintained, and seeds stored under ambient temperature lost the defensive mechanism after six months of storage. Clearly, seeds under the ambient temperature are subjected to more stress than seeds under the refrigerator temperature. Various literature addressed the same issue on the bad effects of storing seeds under the ambient temperature, particularly under humid conditions. This is due to fluctuating temperatures and the relative humidity of the storage environment during the storage period, which greatly affected the germination capacity of the lablab bean seed. It is suggested that less lipid peroxidation occurred in refrigerator-stored seeds with the presence of low temperature and relative humidity throughout the storage period. The association of seed degradation with increased lipid peroxidation and reduced antioxidant enzyme activity during ageing has been confirmed by several studies (Kibinza et al. [34]; Lehner et al. [35]; Xin et al. [36]).

It is very important to balance the production of antioxidant enzymes and accumulation of ROS, which will lead to a minimum level of seed deterioration. It can also be presumed in this study that ROS formation in regulated temperature is much lower and that the amount of antioxidant enzyme activity is sufficient to prevent the harmful effects of ROS produced during storage. In a study by Ushimaru et al. [37], higher peroxidase activity is reported in seeds stored under the ambient storage temperature, which showed an increment in the mechanism of defence and prevented quality loss. Reactive oxygen species (ROS) accumulation and lipid peroxidation are known to cause seed deterioration according to Baily et al. [38].

The study also indicated that deterioration of the lablab bean seeds under the ambient temperature is associated with increased seed moisture content and ultimately causes accumulation of ROS leading to decreased enzyme system activation, increased membrane lipid peroxidation, and genetic damage. It is concluded that the lablab bean seed is more sensitive to adverse weather conditions, as indicated by seed deterioration quality after six months of storage. The results are consistent with earlier reports that stored soybean seeds at the ambient temperature decreased seed germination as the storage period increased, as mentioned by Kandil et al. [39]. In addition, decreased catalase activity is associated with ageing, accompanied by an increase in lipid peroxidation and loss of vigour and viability in sunflowers, according to Bailly et al. [38]. Hence, catalase deficit has served as one of the important markers of oxidative damage. Therefore, the present study agreed that the lablab bean seed is best stored under the refrigerated temperature to retain better storability. The beneficial part of using the refrigerator is it is easily accessible for seed producers and economically friendly compared to large cold rooms with higher costs.

## 4. Conclusions

This study identified the significant effect of both the drying methods and storage temperature on seed quality supported by the retention of seed defense mechanism. It is

evident that the current practice of sun drying is not optimal for drying lablab bean seeds as poor germination, vigour, and seedling performances were recorded. Due to sensitivity of the seeds towards heat, oven drying is not advisable as well. Therefore, drying beads are suggested as they accelerate the drying process and subsequently contribute to higher and faster germination and vigour. The low relative humidity (10–15 RH%) found within closed containers will enable farmers and seed producers to dry seeds to a low moisture content without heat involvement, thus preserving seed quality. In addition, it is advantageous to use drying beads as they are reusable. Thus, it is also a good investment for farmers and seedsman in maintaining seed quality over time.

Seed deterioration under ambient temperature is due to declining antioxidant enzyme activities which affect germination performance. This study found that decreased catalase and peroxidase under high temperatures (27.7–34.2 °C; 74 ± 5% RH), are associated with ageing, accompanied by loss of vigour after six months of storage. In contrast, seeds stored under refrigerator temperatures (2.0–5.0 °C; 25 ± 5% RH) have maintained higher germination and vigour after six months of storage. Furthermore, the results suggested that less lipid peroxidation occurred in seeds stored refrigerated throughout the storage period. The balance between production of antioxidant enzymes and accumulation of ROS will lead to a minimum level of seed deterioration. It can also be presumed that ROS formation in refrigerated temperature is much lower, as the amount of antioxidant enzyme activity is sufficient to prevent the harmful effects of ROS produced during storage. In conclusion, lablab bean seeds should be dried to 10% MC using drying beads and stored refrigerated at 5 °C; with such conditions, no decline in seed quality was observed during the six months adopted for short term storage in this study.

## 5. Future Research Recommendations

The following recommendations can be drawn for further improvement in maintaining lablab bean seed quality. Although various reports have said that lablab beans should be harvested at physiological maturity (30 DAA), PM may not be the best time to harvest the seeds. The initial germination obtained from this study is found to be below the accepted germination for commercial seed production. However, this is achieved without the prior understanding that the purple lablab bean should be harvested at the later stage of harvesting maturity (HM) or in between (35–40 DAA). Hence, further understanding on the development and correct time of harvesting could further improve seed germination to be more than 80%. Further studies can also revise the effect of lower moisture content $\leq$ 8% for lablab beans in order to dry seeds for prolonged storage periods.

**Author Contributions:** Conceptualization, A.M.Y. and U.R.S.; methodology, A.M.Y.; formal analysis, A.M.Y.; investigation, A.M.Y.; data curation, A.M.Y. and U.R.S.; writing—original draft preparation, A.M.Y.; writing—review and editing, A.M.Y.; visualization, A.M.Y. and U.R.S.; supervision, U.R.S. and A.M.; project administration, A.M.Y. and U.R.S.; funding acquisition, U.R.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding authors. The data are not publicly available.

**Acknowledgments:** The authors acknowledge the assistance of the Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia who facilitated the field study and seed quality analysis.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Al-Snafi, A.E. The pharmacology and medical importance of Dolichos lablab (*Lablab purpureus*)—A review. *IOSR J. Pharm.* **2017**, *7*, 22–30. [CrossRef]

2. UC Davis. Drying Beads Help Bangladesh Farmers Access Better Seed. 2017. Available online: https://horticulture.ucdavis.edu/blog/drying-beads-help-bangladesh-farmers-access-better-seed (accessed on 27 April 2021).
3. Das, S.S.; Fakir, M.S.A. Pod growth and seed composition in two genotypes of *Lablab purpureus*. *Legume Res.-Int. J.* **2014**, *37*, 306–310. [CrossRef]
4. TeKrony, D.M.; Egli, D.B. Accumulation of seed vigour during development and maturation. *Basic Appl. Asp. Seed Biol.* **1997**, *30*, 369–384.
5. Golezani, G.K.; Mahootchy, H.A. Changes in seed vigour of faba bean (*Vicia faba* L.) cultivars during development and maturity. *Seed Sci. Technol.* **2009**, *37*, 713–720. [CrossRef]
6. Lassim, M.B.; Chin, H.F. Some trends in the development and maturation of cowpea (*Vigna unguiculata* L.) seeds. *Acta Hort.* **1987**, *215*, 25–30. [CrossRef]
7. Bewley, J.D.; Bradford, K.J.; Hilhorst, H.W.; Nonogaki, H. Longevity, storage, and deterioration. *Seeds* **2013**, 341–376. [CrossRef]
8. Berjak, P.; Pammenter, N.W. From Avicennia to Zizania: Seed recalcitrance in perspective. *Ann. Bot.* **2008**, *101*, 213–228. [CrossRef]
9. Krzyzanowski, F.C.; West, S.H.; França Neto, J.D.B. Drying peanut seed using air ambient temperature at low relative humidity. *Rev. Bras. Sementes* **2006**, *28*, 1–5. [CrossRef]
10. Justice, O.L.; Bass, L.N. Principles and Practices of Seed Storage. In *Agriculture Handbook*; No. 506; Department of Agriculture, Science and Education Administration, U.S. Department of Agriculture: Washington, DC, USA, 1978.
11. Panda, B.B.; Mohapatra, S.; Mallik, S.; Acharya, P. Effect of Tamarind seed mucilage on rheological properties: Evaluation of suspending properties. *Int. Res. J. Pharm. Sci.* **2010**, *1*, 8–10.
12. Nagaveni, P.K. Effect of Storage Conditions, Packing Material and Seed Treatment on Viability and Vigour of Onion Seeds. Ph.D. Thesis, UAS, Dharwad, India, 2005.
13. Jyoti; Malik, C.P. Seed deterioration. *Int. J. Life Sci. Biotechnol. Pharma Res.* **2013**, *2*, 374–385.
14. Singh, A.; Abhilash, P.C. Varietal dataset of nutritionally important *Lablab purpureus* L. sweet from Eastern Uttar Pradesh, India. *J. Data Brief* **2019**, *24*, 103935.
15. ISTA. *International Rules for Seed Testing*; International Seed Testing Association: Bassersdorf, Switzerland, 2016.
16. Abdul-Baki, A.A.; Anderson, J.D. Vigor determination in soybean seed by multiple criteria 1. *Crop Sci.* **1973**, *13*, 630–633. [CrossRef]
17. Kader, M.A. A comparison of seed germination calculation formulae and the associated interpretation of resulting data. *J. Proc. R. Soc. N. S. W.* **2005**, *138*, 65–75.
18. Manju, V.; Kumar, S. Seed leachate conductivity and its correlation with the seed viability and germination of Tnau Papaya Cv. Co8 seeds stored under different environmental conditions. *Int. J. Agric. Sci. Res. IJASR* **2015**, *5*, 127–130. Available online: http://www.tjprc.org/view-archives.php (accessed on 27 April 2021).
19. Aebi, H. Catalase in vitro. *Method Enzymol.* **1984**, *105*, 121–126.
20. Chance, B.; Maehly, A.C. Assay of catalases and peroxidases. *Methods Enzymol.* **1955**, *136*, 764–775.
21. Nassari, P.J.; Keshavulu, K.; Manohar, R.K.; Reddy, A.R. Postharvest drying of tomato seeds to ultra low moisture safe for storage using desiccant (zeolite) beads and their effects on seed quality. *Am. J. Res. Commun.* **2014**, *2*, 74–83.
22. Zaitialia, M.; Atilia, H. Evaluation on the effectiveness of rapid drying method and its effects on chilli (*Capsicum annuum*) seed quality. *Trans. Malays. Soc. Plant Physiol.* **2016**, *23*, 68–73.
23. Ali, M.; Rahman, M.; Rahman, M.; Hossain, M.; Asaduzzaman, M. Effect of Drying Method on Quality of Soybean Seed. *Bangladesh Agron. J.* **2015**, *18*, 53–57. [CrossRef]
24. Hor, Y.L. Storage of field crop seeds under Malaysia conditions. In Proceedings of the National Seed Symposium, Serdang, Malaysia, 4–7 February 1976; pp. 124–134.
25. Muckle, T.B.; Stirling, H.G. Review of the drying of cereals and legumes in the tropics. *Trop. Stored Prod. Inform.* **1971**, *22*, 11–30.
26. Nautiyal, P. Seed and seedling vigor traits in groundnut (*Arachis hypogaea* L.). *Seed Sci. Technol.* **2009**, *37*, 721–735. [CrossRef]
27. Afonso, P.C., Jr.; Corrêa, P.C. Immediate and latent drying effects of bean seeds harvested with different moisture levels. *Ciênc. Agrotecnol.* **2000**, *24*, 33–40.
28. Hanapiah, N.F.H.; Sinniah, U.R.; Yusoff, M.M. Seed Quality of Lablab Bean (*Lablab purpureus*) as Influenced by Seed Maturity and Drying Methods. *Agronomy* **2022**, *12*, 363. [CrossRef]
29. Mohammadi, H.; Soltani, A.; Sadeghipour, H.R.; Zeinali, E. Effects of seed aging on subsequent seed reserve utilization and seedling growth in soybean. *Int. J. Plant Prod.* **2012**, *5*, 65–70.
30. Dona, M.; Balestrazzi, A.; Mondoni, A.; Rossi, G.; Ventura, L.; Buttafava, A.; Carbonera, D. DNA profiling, telomere analysis and antioxidant properties as tools for monitoring ex situ seed longevity. *Ann. Bot.* **2013**, *111*, 987–998. [CrossRef]
31. Pradhan, B.K.; Badola, H.K. Effect of storage conditions and storage periods on seed germination in eleven populations of Swertia chirayita: A critically endangered medicinal herb in Himalaya. *Sci. World J.* **2012**, *2012*, 128105. [CrossRef]
32. Krishna, S.P.; Gejjalagere, H.C. *Advancement of Biodegradable Polysaccharide Nanocarriers for Delivery of Herbal Extracts and Bio-Actives*; CRC Press: Boca Raton, FL, USA, 2018; pp. 191–205.
33. Pukacka, S.; Ratajczak, E. Antioxidative response of ascorbate-glutathione pathway enzymes and metabolites to desiccation of recalcitrant Acer saccharinum seeds. *J. Plant Physiol.* **2006**, *163*, 1259–1266. [CrossRef]
34. Kibinza, S.; Vinel, D.; Come, D.; Bailly, C.; Corbineau, F. Sunflower seed deterioration as related to moisture content during ageing, energy metabolism and active oxygen species scavenging. *Physiol. Plant.* **2006**, *128*, 496–506. [CrossRef]

35. Lehner, A.; Mamadou, N.; Poels, P.; Come, D.; Bailly, C.; Corbineau, F. Changes in soluble carbohydrates, lipid peroxidation and antioxidant enzyme activities in the embryo during ageing in wheat grains. *J. Cereal Sci.* **2008**, *47*, 555–565. [CrossRef]
36. Xin, X.; Tian, Q.; Yin, G.; Chen, X.; Zhang, J.; Ng, S.; Lu, X. Reduced mitochondrial and ascorbate-glutathione activity after artificial ageing in soybean seed. *J. Plant Physiol.* **2014**, *171*, 140–147. [CrossRef] [PubMed]
37. Ushimaru, T.; Kanematsu, S.; Katayama, M.; Tsuji, H. Antioxidative enzymes in seedlings of Nelumbo nucifera germinated under water. *Physiol. Plant.* **2001**, *112*, 39–46. [CrossRef] [PubMed]
38. Bailly, C.; Bogatek-Leszczynska, R.; Côme, D.; Corbineau, F. Changes in activities of antioxidant enzymes and lipoxygenase during growth of sunflower seedlings from seeds of different vigour. *Seed Sci. Res.* **2002**, *12*, 47. [CrossRef]
39. Kandil, A.A.; Sharief, A.E.; Sheteiwy, M.S. Effect of seed storage periods, conditions and materials on germination of some soybean seed cultivars. *Am. J. Exp. Agric.* **2013**, *3*, 1020.