Effect of Storage Periods on Physical Quality and Seed Vigor of Four Varieties of Sorghum (*Sorghum Bicolor* [L.] Moench)

Paul B. Timotiwu¹, Eko Pramono¹*, Agustiansyah¹ & Ni Wayan Ayung Surya Asih²

¹ Department Agronomy and Horticulture, University of Lampung, Bandar Lampung, Indonesia
² Department Agrotechnology, University of Lampung, Bandar Lampung, Indonesia

* Eko Pramono, E-mail: pramono.e61@gmail.com

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Abstract

The availability of good physical quality and high seed vigor is expected to occur with good handling of storage. The purpose of this study was to determine the effect of two storage periods [10 months (p1) and 12 months (p2)] and different sorghum varieties of Kawali (V1), Talaga Bodas (V2), Super-1 (V3), and Pahat (V4) on the physical quality and sorghum seed vigor. This experiment was conducted at the Laboratory of Seeds and Plant Breeding, Faculty of Agriculture, the University of Lampung during November 2015 to November 2016. The experiment was laid out in Randomized Complete Block Design (RCBD) with two replications. The variable damaged seed was higher by 24.19% compared to seeds stored for 10 months at 14.73%. The percentage of total normal seedling after store 12 months (59.50%) was lower than 10 months (78.00%). Speed germination of seed after store 12 months (24.58%/day) was lower than the stored 10 months (36.47%/day). Tetrazolium test for vigor showed no different results from germination test results. The variety that has the best seedling vigor was Super-1 indicated by primary root length and dry weight of normal seedlings.

Keywords

physical quality, seed, sorghum, storage period, vigor

1. Introduction

*Sorghum (Sorghum bicolor* [L.] Moench)* is a plant similar to corn in terms of agronomic and nutritional value (Neto et al., 2017). However, the requirements in terms of growth, sorghum is superior because it can adapt to drought and low soil fertility (Borba et al., 2012). Sorghum can be used for alternate food sources of raw materials bioethanol industry (bars) as well as animal feed. The nutritional content of sorghum is comparable to corn and rice, having protein content to the tune of 10-11%. Sorghum has a high nutrient content that is 339 calories and 11.3% protein/100 grams of seeds in seeds and 12.8% crude protein in the vegetative to meet the nutritional needs of dairy cows and beef cattle fattened (USDA,
2011). In addition, the sorghum is also used as animal feed in developed countries such as Japan, USA and Europe. Lack of availability of quality seeds led to a decline in production caused by the use of low-quality seeds—and adaptation in the field is reduced (Jyoti & Malik, 2013). Availability of quality seeds is related to seed storage. According to Goftishu and Belete (2014), good handling during storage period can minimize physical damage to the seed, especially seed quality during storage, so the seeds can be stored for a long storage period.

Bad handling of seed storage for the long term would make the seed deteriorate. Deterioration seed is a process that occurs naturally and cannot be stopped. Vigor seeds were decreased during storage (Akter et al., 2014). Seed storage in tropical areas such as Indonesia had become a main problem due to the problem of high humidity and temperature fluctuations. Each lot of seed has a different lifespan at certain storage period. According to Bortey et al. (2016), seed storage period may affect the viability of seeds, as the reduction in seed viability is directly proportional to the increase of time. This is because it allows the ripening embryo storage period and further accumulation of food that lasts for storage before germinating, these activities led to an increase in the metabolic processes in the seed. As a result, the seed has decreased viability and cannot germinate optimally, because energy has been used in the metabolic process (Badawi et al., 2017). In addition to a storage period of seed, seed viability was also influenced by seed storage environments such as temperature and relative humidity (Strelec et al., 2010). The seeds stored at low temperature storage germinated higher than those stored at high temperatures storage-in all storage period (Mbofung, 2012). It because the seeds are stored in high temperatures increase the respiration rate and enzyme activity resulting in the overhaul of food reserves before the seeds germinate, the seeds decreased vigor and physical quality of seed. The study was conducted to determine the effect of storage period and varieties on the physical quality and vigor of sorghum seed.

2. Method

2.1 Material and Tools

This study was conducted at the Laboratory of Seed Technology and Plant Breeding, College of Agriculture University of Lampung Indonesia, from November 2015 to November 2016. Sorghum seed varieties in this study were Kawali, Talaga Bodas, Super-1, and Pahat, water ion-free, compact disc (CD) and straw paper, 2,3,5-triphenyl tetrazolium chloride (Merck, Pte. Singapore). The tools were used in this research are plastic containers, staples, stationery, rubber, ruler, plastic wrap glue, petri dish, scalpel, tray, labels, seed blower, oven, counter seed type Seedburo 801 count- A-PAK (Seedburo, IL, USA), electric scales type Scout Pro, scales analytical type (Cole Parmer PA USA), electroconductivity meter type series WTW pH/Cond 720, grain moisture tester type GMK 303 RS (WTW, Weilheim, Germany), germination IPB type 71-2A (IPB, Bogor, Indonesia) and plastic cups.

Two treatments were arranged factorially in a randomized complete block design (RCBD). The first factor was the storage period (P), which consisted of a storage period of 10 months (P1) and 12 months period (P2). The second factor was the variety (V) consisted of Kawali (V1), Talaga Bodas (V2), Super-1
(V3) and Pahat (V4). There were 8 combined treatment in each trial and each treatment consisted of two replications, in order to obtain 16 units of experimental units. Homogeneity of variance of data was tested by Bartlett’s test and non-additivity of model was tested by Tukey. The difference between the means was tested using Honestly Significant Difference (HSD) at $\alpha 0.05$.

2.2 Implementation Research

2.2.1 Harvesting
Sorghum seeds obtained from Marhain Village, Sub-District Anak Tuha, District of Central Lampung. The seed that had been collected, then it dried and cleaned using a seed blower.

2.2.2 Packaging
A number of 200 grains of cleaned sorghum seeds were put in plastic cup container and then placed into the tray, arranged and glued.

2.2.3 Storage
The seeds in plastic cup container were stored in a room with temperature $\pm 180^\circ$C and humidity $\pm 48\%$ for 10 and 12 months of storage period.

2.3 Physical Quality

2.3.1 Seed Damaged
It was obtained from sorting of seed based on their physical appearance visually as by ISTA procedure. It was characterized by the seed size less than half, crack seeds, potholes and empty due to weevil, embryo of seeds were not intact and wrinkles. It was expressed as a proportion of the total number of samples of seeds of 200 grains seed to get percentage of damage seeds (Gofdishu & Belete, 2014).

2.3.2 Seed Moisture
Seed moisture content measurements were performed using the moisture tester. The measurement was done by taking five-grain sorghum seeds at random and be repeated twice.

2.4 Seed Vigor

2.4.1 Electrical Conductivity (EC)
Electrical Conductivity (EC): 25 grains of sorghum seeds soaked in 50 ml of deionized water and then covered with plastic and allowed to stand for 24 hours. The electrical conductivity of water immersion of seeds was measured using a conductivity meter (ISTA, 2010). Then, the electrical conductivity was calculated using the formula:

$$\text{Conductivity (}\mu\text{S. Cm}^{-1}) = \text{Conductivity of bath water} - \text{blank}$$

2.4.2 Germination Test
The method used for germination test based on ISTA (2010) namely rolled paper test. Seed germination test consisted of Germination Speed’s test in which 25 grains of sorghum seed every trial were put on two layers of moist straw paper then covered with two layers of it, afterward it was rolled and it must be in the moist condition. All the rolled papers were placed in a germinator of IPB type 71-2A to room temperature ($26 \pm 0.40^\circ$C) (ISTA, 2010). Observation consists of Germination Speed (SG) was a cumulative total percentage of normal seedling daily since observation 2 to 5. Germination speed was calculated by the
following formula (Copeland & Donald, 2005):

\[ SG = \frac{NS_1}{d_1} + \frac{NS_2}{d_2} + \frac{NS_3}{d_3} + \ldots + \frac{NS_n}{d_n} \]

\(SG\) = speed of germination (%/day); \(NS\) = The percentage of normal seedlings at each observation (%); \(d\) = day of observation; \(n\) = days of final count.

2.4.3 Total Normal Seedlings

Total Normal Seedlings were the number of normal seedling calculated from observations of day 2 until day 5. Total normal germination percentage was calculated using the following formula (ISTA, 2010):

\[ TNS = \frac{\sum NS_i}{n} \times 100\% \]

\(TNS\) = total normal seedlings (%); \(\sum NS\) = number of total normal seedlings that grow at the end of the observation; \(i\) = days of observation to-2, 3.4 and 5, \(n\) = number of seeds germinated was 25.

2.4.4 Dead Seeds

Dead seeds were a seed that did not show a sign of germination (ISTA, 2010). Number of dead seeds were calculated on the 5th day of observation.

2.4.5 Normal Strong Seedlings

Normal strong seedlings were measured from Seedling Vigor Classification Test. Twenty-five grains of sorghum seed every trial were put on two layers of moist frosted paper, covered with two layers of moist frosted paper, afterward it was rolled, then placed in a germination IPB type 71-2A at room temperature (26 ± 0.4°C). Observations consisting of Normal Strong Seedling and Weak normal seedling at four days after germinating. Normal strong seedlings were the seedling that has a strong of the shoot and the primary root more than 4 cm.

2.4.6 Normal Weak Seedling

Normal Weak Seedling was a normal seedling characterized by small shoot and primary root of 4 cm or less. It was based on Copeland and McDonald (2005) criteria for peanut seedling.

2.4.7 Shoot Length of Normal Seedling

Shoot Length of Normal Seedling was measured by five samples of normal seedlings that were taken at random and it was measured the length of the shoot that grew at the base of the seeds to the tip of the shoot.

2.4.8 Primary Root Length of Normal Seedling

Primary Root Length of Normal Seedling was obtained by measuring the length of primary root from the base to the tip of the root as follow as described by Kandil et al. (2013) and AOSA (2010).

2.4.9 Dry Weight of Normal Seedling

Dry Weight of Normal Seedling was measured by using five samples of normal seedling which has been dried in the oven for 3x24 hours at temperature of 80°C (Copeland & McDonald, 2005).

2.4.10 Tetrazolium Test (TZ)

A number of 25 grains of sorghum seeds were moistened in moist straw paper for 18 hours. Then the
seeds were vertically split into two halves with embryos seen as the right and left. Then, the seed was soaked within 1.0% tetrazolium solution and put into the incubator with temperature of 30°C for 18 hours. Observations were made to see viable and non-viable of the seeds (ISTA, 2010).

3. Result

3.1 Summary of Analysis of Variance

Analysis of variance showed that the differences in the physical storage period affecting the quality of sorghum seeds are indicated by the percentage of damaged seeds, but no difference in variable seed moisture content. There were no differ in physical quality, either damaged seeds and moisture content of the seeds for all varieties. In sorghum, seed vigor showed different results due to differences in storage period, except for the normal weak seedlings.

Difference of seed vigor on varieties of sorghum was shown by the variable electrical conductivity, primary root length of normal seedling and dry weight of normal seedling. Based on the results there is no interaction between storage period and the difference sorghum varieties were used for all variables were observed (Table 1).

Table 1. Summary of the Analysis of Variance the Effect of Storage Seriod (P) and Variety (V) on the Physical Quality and Sorghum Seed Vigor

| No. | Observation variables                                         | Treatment | p   | V   | P x V |
|-----|---------------------------------------------------------------|-----------|-----|-----|-------|
| A.  | Physical quality                                              |           |     |     |       |
| 1   | Damage seed (%)                                               | **        | ns  | ns  |       |
| 2   | Seed moisture content (%)                                      | ns        | ns  | ns  |       |
| B.  | Vigor seed                                                   |           |     |     |       |
| 3   | Electrical conductivity (μS.cm⁻¹)                             | **        | *   | ns  |       |
| 4   | Normal strong seedlings(%)                                    | *         | ns  | ns  |       |
| 5   | Normal weak seedlings (%)                                     | ns        | ns  | ns  |       |
| 6   | Shoot length of normal seedling (cm.seedling⁻¹)               | **        | ns  | ns  |       |
| 7   | Primary root length of normal seedling (cm.seedling⁻¹)       | **        | *   | ns  |       |
| 8   | Dry weight of normal seedling (mg.seedling⁻¹)                | **        | *   | ns  |       |
| 9   | Germination Speed (%/day)                                     | **        | ns  | ns  |       |
| 10  | Total normal seedling (%)                                     | **        | ns  | ns  |       |
| 11  | Dead seed (%)                                                | *         | ns  | ns  |       |

Note. P = period of storage; * = significantly different at α = 0.05; v = varieties; ** = very significantly different at α = 0.01; V x P = interaction effect of P and V; ns = not significantly different at α = 0.05
3.2 Effect of Storage Period on the Physical Quality and Vigor of Sorghum Seed (Sorghum Bicolor [L.] Moench.)

The results showed that all varieties were stored at storage room with temperature of ± 18°C and RH ± 48%, the percentage of damaged seeds were higher for 12 months storage period than 10-month storage period, by 24.19% and 14.73%, respectively. Those results were in line with statement of Goftishu and Belete research (2014), the final percentage of the damaged seed with the highest recorded of 29.33 ± 2.23%. In this experiment, the percentage of moisture content of seeds stored for 10 months of 8.90% was not different from the 12 months, 8.98% (Figure 1). This was presumably that the seeds were stored in a cold room, seed moisture content depends on the humidity of the surrounding air, if the air humidity increases, the seed moisture content increases too, and if the humidity is low, the seed moisture content would decrease (Mbofung et al., 2013).

The effect of storage period on seed vigor was the increase of electrical conductivity of the soaking water of the seeds, from 48.66 μS.cm⁻¹ in the seeds that stored for 10 months and 61.89 μS.cm⁻¹ from those stored for 12 months. There was difference of 13.23 μS.cm⁻¹. Naguib et al. (2011) stated that the increasing storage period from 0 to 18 months led to an increase in the value of electrical conductivity of a wheat seed 16.03 to 52.02 μS.cm⁻¹. During storage has suffered deterioration as indicated by electrolyte leakage that increase the value of electrical conductivity in soaking water. Moreover Naguib et al. (2011), increasing storage period also resulted in germination and dry weight decreased, at the end of the observation of the initial germination percentage decreased from 82.3% (0 months) to 53.6% (18 months), followed by the number of dead seeds. The statement was in line with the increase in the percentage of dead seeds which from 25.31% (at 10 months) to 30.16% (at 12 months) (Figure 2).
Figure 2. Electrical Conductivity (A), Dead Seed(B), Normal Strong Seedlings (C), Shoot Length of Normal Seedlings (D), Primary Root Length of Normal seedling (E), Dry Weight of Normal Seedling (E), Speed Of germination (G), and Total Normal Seedlings (H). Mean Value Followed by the Same Letters was Not different at Α 0.05 Refers to Tukey Test of HSD

In normal strong seedlings, shoot length of normal seedlings, primary root length of normal seedlings,
dry weight of normal seedlings, the speed of germination and total normal seedlings decrease could be seen in Figure 2. Kandil et al. (2013) reported that root length, shoot length and dry weight of normal seedling decreased after being stored for 12 months when compared to the 3,6, and 9 months stored period of soybean seeds. Increased storage period on seed wheat from storage of 3,6 and 9 months significantly affect the viability and quality of seeds, the germination percentage was lowest in 9 months. It showed that seed deterioration during storage likely to result in membrane damage, enzymes, proteins, and nucleic acids that eventually led to lost percent germination and the death of the seed (Badawi et al., 2017).

3.3 The Effect of Different Varieties on the Physical Quality and Vigor of Sorghum (Sorghum Bicolor [L.] Moench.)

The different varieties on seed vigor showed different results in electrical conductivity with the highest value of the varieties Super-1 amounted to 64.65 \( \mu \text{S.cm}^{-1} \) and Kawali amounting to 62.84 \( \mu \text{S.cm}^{-1} \) when compared to varieties Pahat of 46.29 \( \mu \text{S.cm}^{-1} \) but no different from Talaga Bodas 47.33 \( \mu \text{S.cm}^{-1} \) (Figure 3). Increased electrical conductivity signifies a decrease in seed vigor, as well as the characteristic that the seeds are regressing as indicated by increasing electrolyte leakage (Akter et al., 2014).

![Figure 3. Electrical Conductivity of Four Varieties of Sorghum. Mean Value Followed by the Same Letters Was not Different at \( \alpha 0.05 \) Refers to Tukey Test of HSD](image)

In the primary root length of normal seedling showed Talaga Bodas has the longest primary root amounted to 9.23 cm.seedling\(^{-1}\), but not different than Super-1 amounted to 8.54 cm.seedling\(^{-1}\) compared with Kawali of 7.01 cm.seedling\(^{-1}\) supported by dry weight of normal seedling indicates the Talaga Bodas and Super-1 has the highest dry weight respectively by 6.99 mg.seedling\(^{-1}\) and 6.72 mg.seedling\(^{-1}\) compared to Kawali of 4.92 mg.seedling\(^{-1}\). Super-1 has the best seedling vigor because the Super-1 is a variety that has the highest electrical conductivity value is an indicator of seed deterioration (Figure 3) but does not cause primary root length and dry weight of normal seedling low. So, that Super-1 did not differ from Talaga Bodas (Figure 4) as well as the evaluation of vigor through Tetrazolium test shows the
percentage of viable seeds in Super-1 is still high (Table 2).

Root length and dry weight difference may be due to genetic differences and biochemical composition of each variety causing differences in deterioration rate and decreased vigor (Kandil et al., 2013). High dry weights showed that the use of reserves in the seed is very efficient because the seeds that have a high vigor can transmit raw materials to the embryo shaft quickly so that the accumulation of dry weight increases. The size of the seeds of each variety can also affect the dry weight of normal seedling because the seeds with large sizes were seeds that have large food reserves. If the vigor seeds were high, so the use of food reserves was very efficient to use during germination.

Table 2. Evaluation of Sorghum Seed Vigor through Tetrazolium Test

| Varieties     | Storage period |
|---------------|----------------|
|               | 10 months (%)  | 12 months (%) |
| Kawali        | -              | 56            |
| Talaga Bodas  | 80             | 68            |
| Super-1       | 80             | 68            |
| Pahat         | NA             | 68            |

The number of dead seed percentage increased with increasing storage period. The percentage of dead seeds was highest in soybean seeds stored in the storage period of 60 days after the stored, which is the longest storage time of the study (Akter et al., 2014). Increased temperature and storage period causes percentage germination seeds, the number of normal seedling and enzyme activity decreased (Azadi & Younesi, 2013). The statement was in line with the number of nonviable seeds in the tetrazolium test (Figure 5).
Figure 5. Evaluation of Sorghum Seed Vigor through the Tetrazolium Test, the Seed with Full Red Embryo Are Viable Seed (A); the Seed with Bruises (B) and White Spots (C) Are Non Viable Seeds

4. Conclusion

All varieties of sorghum were stored at storage room with temperature of ± 18°C and RH ± 48% for 12 months yielded damage seed was higher than stored for 10 months, by 24.19%, 14.73%, respectively. Seed vigor as shown by the percentage of total normal seedling stored at storage room with temperature of ± 18°C and RH ± 48% for 12 months was lower than stored at storage room for 10 months, by 59.50%, 78.00%, respectively. Also, the speed germination of seed after store 12 months was lower than it stored at storage room for 10 months, by 36.47%/day, 24.58%/day, respectively. Sorghum variety that has the best seedling was Super-1. It was indicated by root length and dry weight of normal seedlings.

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