



Viability and vigor of cocoa seed (*Theobroma cacao* L.) to some levels of water content and old period of save

Saiful Bahri*, Syamsuddin, Syafruddin

Department of Agroecotechnology, Faculty of Agriculture, University of Syiah Kuala, Darussalam, Banda Aceh, Indonesia

Article published on August 30, 2018

Key words: Electrical conductivity, Free fatty acids, Viability, Vigor.

Abstract

Propagation of cocoa plants is generally still done generatively, so the handling to produce good quality seed is very important to note. Problems constraining cocoa seed procurement include: The distance between the seed source and the development area is far enough, the delivery facilities are inadequate, when the seeds are available not in accordance with the time of demand by the user, and the viability of cocoa seeds are rapidly declining. This study aims to determine the optimal cocoa water content for storage, to know the optimal storage of cocoa seeds and to know the effect of interaction between water content treatment and storage duration on cocoa seed viability and vigor. The design used in this research is Completely Randomized Design (RAL) factorial pattern with 3 replications. The results showed that the level of water content influenced the viability and vigor of cocoa seeds. The decrease in viability and vigor of the seeds is closely related to the moisture content of the seed. Storage of cocoa seeds at moisture content of 35-38% can suppress the rate of decline of seed. The length of the shelf period affects the viability and vigor of cocoa seeds. The longer the seed is stored, the lower the viability and the vigor. Viability and vigor of seeds even in the 6 week saving period have already shown seed death. There is an interaction between the moisture level and the length of the shelf period on the viability and vigor of cocoa seed. The rate of decline of cocoa seeds in each saving period depends on the degree of moisture content of the seed. Up to the 6-week saving period, viability and vigor of cocoa seeds can be maintained at a 25-28% moisture level.

*Corresponding Author: Saiful Bahri ✉ saifulagt13@gmail.com

Introduction

Cocoa (*Theobroma cacao* L.) is one of the most important plantations to be developed by both large estates and smallholders. The development of cocoa area has been done in several provinces covering the islands of Sumatra, Kalimantan, Sulawesi and Papua. The total area of cocoa plantations in Indonesia in 2008 of 1,425,217 ha increased to 1,732,954 ha in 2012 (Directorate General Plantation, 2013).

Propagation of cocoa plants is generally still done generatively, so the handling to produce good quality seed is very important to note. Superior cocoa seed is only produced by seed producers appointed by the government through the Ministry of Agriculture. Currently, the need for problem seed seeds that constrains cocoa seed procurement is the location of seed sources with extensive expansion areas, inadequate delivery facilities of semi-limited air transport facilities in cocoa development centers, thus requiring relatively long time during delivery this can decrease the quality of the seed, especially the physiological quality (Adelina and Maemunah, 2004).

Several factors affecting the viability of recalcitrant seeds include moisture content of seeds, humidity, storage space temperature, container, and storage period. Seed water content greatly determines the viability of seeds to maintain shelf life hence the water content of seeds is kept high. The longer the seeds stored, the observed water content parameters that are affected by the long storage factor indicate a decrease. The occurrence of the decline is caused by the decrease in food reserves in the seeds with increasing length of storage. This indicates that as long as the seeds are stored there is a process of respiration in the seeds, so the food reserves contained in the cotyledons used as energy reserves in the process of growth of the seeds are further reduced (Justice and Bass, 2002).

Problems with the current procurement of cocoa seeds include: (a) The distance between seed sources and development areas far enough, (b) inadequate delivery means, (c) when seeds are available not in accordance with the timing of demand by users, and

(d) viability of rapidly declining cocoa seeds. Based on the above problem, it is necessary to research about the influence of moisture content and storage duration on cocoa seed viability and vigor.

Material and methods

This research was conducted from July 2014 until April 2015 at the Laboratory of Seed Science and Technology Faculty of Agriculture, Syiah Kuala University of Darussalam Banda Aceh. The instruments used in this research are analytical scales, a set of spinning power test apparatus, oven, desiccator, germinator, conductor, biuret, petri dish, measuring cylinder, biuret, test tube, air-conditioned storage room, hoe, mesh sieve. The materials used in this research are cocoa seeds of varieties of TSH 858 from coffee and cocoa stem top garden of Tangse Pidie Forestry and Plantation. Seed taken in the form pithy seeds in 2/3 the middle of the fruit. Flesh or pulp is cleaned with careful ash to prevent seeds from physical damage.

The seeds that have been cleaned are then measured the initial moisture content by placing in 10 petri dish containers, then weigh the initial weight to get the wet weight, then the cup containing the seeds is diesel for 3x24 hours at 105⁰C, after which it is cooled in desiccator for 15 minutes then weighed dry weight. Seed water content is calculated by the formula:

$$\text{Moisture content (\%)} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100 \%$$

The determination of the seed moisture content for the treatment is done by drying using an air-conditioned room. To obtain the seed moisture level in accordance with the drying experiments carried out for a certain time each desired moisture content (20-23%, 25-28%, 30-33% and 35-38%).

Seeds are stored in clear plastic bag containers of 10x15cm without being perforated (airtight). Each bag contains 50 seed, the seed is stored according to the length of the shelf period in accordance with the experimental stage. The calculation of the duration of the shelf period starts from the first day of the decrease in water content in accordance with the level

of experiment conducted. Seed saving space is a room equipped with temperature and humidity (AC room).

Observation parameters

1. Maximum Growing Potential

Testing the maximum growth potential is done by planting cocoa seeds on the sand media as much as 25 grains in the container box germination. Growing potential is determined by the number of seeds that grow at the last observation (14 days after planting). Criteria of seeds that grow is a seed that shows symptoms of growth, meaning that the root or plumula exit through the skin of seeds of sprouts.

Maximum Growing Potential (%)

$$= \frac{\sum \text{seeds showing symptoms of growing}}{\sum \text{seeds planted}} \times 100 \%$$

2. Sprout Power

The germination rate indicates the number of normal seeds produced by the seeds under optimum environmental conditions. Germination rate is calculated based on the percentage of seeds that germinate normally from the amount of seeds that are added. A total of 25 seeds from each treatment were added to the germination media medium. Seed germination was calculated on the 7th day and the 14th day after the seeds were added. The normal seedling criteria are healthy growing hypocotyl with a length of at least twice the length of the seed, open cotyledons and growing epicotyl healthy. Germination power calculation is done with the following formula:

Sprout Power (%)

$$= \frac{\sum \text{normal germinated seeds (I + II)}}{\sum \text{seeds planted}} \times 100 \%$$

3. Growth Speed

Observation of the growing speed is done every day starting from day one. The growth rate is calculated based on the sum of the normal seedling percentages grown on days 1 to 14 divided by etmal (1 etmal = 24 hours), or by the following calculations:

Growth Speed (%/etmal)

$$= \frac{\% \text{ Normal Sprouts}_1}{\text{Etmal}_1} + \frac{\% \text{ Normal Sprouts}_2}{\text{Etmal}_2} + \frac{\% \text{ Normal Sprouts}_3}{\text{Etmal}_3} + \dots + \frac{\% \text{ Normal Sprouts}_{17}}{\text{Etmal}_{17}}$$

4. Diameter of Seed Stems

The seed stem diameter was measured when the seedlings were 60 HST.

5. Weight Dry Seeds

The dry weight of seedlings is determined on the seeds of 60 HST. The seedlings are dried in an oven with a temperature of 60°C for 72 hours, after which it is weighed and expressed in grams.

6. Electric Conductivity

Electrical conductivity measurements are made by measuring the immersion water content of 5 seeds for each experimental unit which has been immersed in ion free water for 24 hours. After 24 hours seed removed and immersion water is measured its electrical conductivity by means of conductivity meter. As the blanks are used 50 ml free ion water which has been stored in a glass of immersion for 24 hours. The electrical conductivity measurements for blanks use the same method as the measurement of treated water with treatment. The value of electrical conductivity is derived from the reduction of the conductivity of the water immersion water with the value of the electrical conductivity of the blank.

7. Determination of Free Fatty Acids

The content of free fatty acids is done by titration. The extraction fat, weighed 1 g, was then fed into the erlenmeyer flask and 25 ml of alcohol solution was added: benzene (1: 1). Furthermore, a drop of the phenolphthalein indicator is added and titrated with 0.1 N Na OH. The titration is discontinued when the color change becomes pink. The content of free fatty acids is calculated using the formula:

Free Fatty Acids

$$= \frac{V \text{ Na OH} \times N \text{ Na OH} \times \text{Fatty acid molecular weight}}{\text{Weight sample (gr)} \times 1.000} \times 100\%$$

Information:

V Na OH: Volume of Na OH required

N Na OH : Normality of Na OH (0.1N)

Molecular weight of linoleic acid: 280

The study was designed as a Completely Randomized Design factorial pattern with 3 replications.

Data were analyzed statistically with ANOVA (Analysis of Variance) technique. The analysis of the effect of moisture level and duration of storage period was determined by using F test. The significant difference between treatments was tested by BNJ method at 5% probability level.

Results and discussion

The Influence of Water Levels and the Old Period of Save on Viability and Seed Vigor

The result of variance analysis showed that the moisture level, the shelf period and the interaction both had a very significant effect on viability and seed vigor. The average value of maximum growth potential, germination rate, relative growth rate, seed stem diameter, and dry weight of seedlings can be

seen in Tables 1, 2, 3, 4, and 5. Based on the observations in Table 1 indicating the potential seed growth without storage at moisture content 20-23%, 25-28% and 35-38% showed no significant difference. But in the other treatment there is a decrease in the potential for seed growth in line with the length of the shelf period. At 30-33% moisture content and 6 weeks of storage time the seeds have died and at every level of water content and storage time of 8 weeks turns out that all the seeds have died.

The results of respiration in the shelf life of seeds in the form of heat and water vapor. The heat that arises as energy scattering in the seeds that should be stored during storage can directly cause viability and vigor of the seeds to decrease (Purwanti, 2004).

Table 1. Mean maximum potential growth value due to the influence of interaction between moisture level and cocoa seed saving period.

Moisture Content	Periods Save Seeds				
	0	2 weeks	4 weeks	6 weeks	8 weeks
..... Growing Potential (%)					
20-23%	100 cA (89.96)	35 bA (36.22)	2.50 abA (7.55)	5 bA (10.66)	0 aA (0.64)
25-28%	100 cA (89.96)	47.50 bA (43.54)	45 bB (42.02)	30 abA (32.20)	0 aA (0.64)
30-33%	96.67 bA (83.82)	27.50 abA (31.60)	5 aAB (10.66)	0 aA (0.64)	0 aA (0.64)
35-38%	97.50 cA (82.63)	37.50 bA (37.74)	17.50 bAB (24.38)	5 abA (12.92)	0 aA (0.64)
BNJ 0.05			32.03		

Description: The numbers followed by the same letter (lower case in the same row, capital letters in the same column) show no significant difference at the 5% probability level; data transformation.

The existence of differences in the potential for seed growth at each level of water content for each period of shelf, indicates that the response to the potential for seed growth of different water content for each period of storage period. Based on the research results can be seen that the water content of seed has a positive relationship with the potential to grow. This means that the higher the moisture content of the seed, the higher the potential grows. According to Prawoto (2008), cocoa seed storage

areas ideally require room temperature 18-30°C, and 100% relative humidity with 50% seed water content. Table 2 shows that the germination of seed groups stored at the moisture level of 35-38% did not show any significant difference although the storage period was different. Just as the potential grows at 30-33% moisture content and 6 weeks of storage time indicates that the seeds have died and at every level of water content and storage time of 8 weeks it turns out that all the seeds have died.

Table 2. Average germination values due to interaction effects between moisture levels and cocoa seed saving period.

Moisture Content	Periods Save Seeds				
	0	2 weeks	4 weeks	6 weeks	8 weeks
..... Sprout Power (%)					
20-23%	70 cA (56.77)	22.50 bA (28.28)	2.50 aA (7.55)	2.50 aA (7.55)	0 aA (0.64)
25-28%	77.50 cA	40 bA	40 bB	17.50 bB	0 aA

Moisture Content	Periods Save Seeds				
	0	2 weeks	4 weeks	6 weeks	8 weeks
 Sprout Power (%)				
	(62.30)	(39.20)	(38.84)	(23.61)	(0.64)
30-33%	72.50 cA	20 bA	2.50 aA	0 aA	0 aA
	(58.36)	(26.55)	(7.55)	(0.64)	(0.64)
35-38%	85 cA	25 bA	17.50 bB	2.50 aA	0 aA
	(67.38)	(29.99)	(23.61)	(7.55)	(0.64)
BNJ 0.05			15.78		

Description: The numbers followed by the same letter (lower case in the same row, capital letters in the same column) show no significant difference at the 5% probability level; data transformation.

One of the requirements of the seed is said to have good quality if the sprouts can reach a minimum of 80% (Minister of Agriculture Regulation, 2013). The interaction between the moisture level 35-38% and without storage process is still able to maintain the germination remains high is 85%. The high sprouts are influenced by high water content, because high water content will help maintain the recalcitrant cell structure (Tambunsaribu *et al.*, 2017). According Sumampow (2011) recalcitrant seed decline caused by the decrease in water content can be physiologically indicated that the germination rate is decreased. During storage, the seeds will experience aging and deterioration (deterioration). Reversed seeds,

increased respiration rates that cause a reduction in food reserves, the accumulation of metabolites resulting from a shakeup of food reserves, can lead to "starvation" of meristem tissues.

Recalcitrant seeds do not experience drying at the time of cooking, regardless and dispersed with relatively high water content conditions ranging from 30-70%. Under these conditions, metabolism remains active and the process toward germination persists even in a resting state. When the seed is dried, subcellular changes begin to occur in the event of desiccation and decrease in water content, resulting in seed viability also decreases (Esrita, 2009).

Table 3. Average relative growth rate of influence of interaction between moisture level and cocoa seed saving period.

Moisture Content	Periods Save Seeds				
	0	2 weeks	4 weeks	6 weeks	8 weeks
 Speed Grows Relatively (%/etmal)				
20-23%	56.82 cA	15.35 bA	2.48 aA	3.03 aA	0 aA
	(48.91)	(23.03)	(7.51)	(8.30)	(0.64)
25-28%	62.32 cA	29.59 bA	32.29 bB	15.29 bB	0 aA
	(52.21)	(32.90)	(34.11)	(21.75)	(0.64)
30-33%	56.93 cA	15.90 bA	1.82 aA	0 aA	0 aA
	(48.96)	(23.48)	(6.45)	(0.64)	(0.64)
35-38%	70.58 cA	19.14 bA	9.13 abA	2.75 aA	0 aA
	(57.14)	(25.93)	(17.27)	(7.91)	(0.64)
BNJ 0.05			13.24		

Description: The numbers followed by the same letter (lower case in the same row, capital letters in the same column) show no significant difference at the 5% probability level; data transformation.

Table 3 shows that the growth rate of seeds at the moisture content of 35-38% and without storage is the highest growth rate of 70.58%, but it does not show any significant difference even in different storage periods. The same is true of the moisture levels and other storage periods that each value of growing velocity does not show a significant difference this means that each treatment has the same effect on the rate of decline of the seed.

The low speed of growing seeds suggests that the quality of the seeds has been low, due to the rate of deterioration of the seed that continues during storage. The loss of seed quality during storage is due to the decrease of seed content due to evaporation as long as the seed is stored. Water temperatures and seeds play a very important and fundamental role in determining the viability status of seeds during storage (Khrisnan *et al.*, 2005).

The storage capacity or the ability of the seed to be stored is determined by the initial moisture content of the seed. The water content for storage is limited to its critical water content (Yuniarti and Djaman, 2015).

The growing speed of seeds that are directly grown (without storage) is higher than after the seeds undergo the storage process. It shows that under these conditions the seed germination is perfect as a good indicator of seed vigor (Rohandi and Widyani, 2016). The rate of growth slows down with decreased germination and increasing storage time due to decreased food stocks in the seeds, including moisture content as a material of metabolic processes (Sulaiman *et al.*, 2010).

Table 4 shows the interaction between moisture levels and seed saving period, but did not show any significant difference in each treatment. The largest seed stem diameter was found in the interaction of 20-23% moisture content with no seed storage process that is 35.70 mm and continues to decrease with the length of seed saving period. This indicates that the length of the shelf period can decrease the viability, causing a decrease in seedling power with the smaller diameter of the stem indicator. According to Rahardjo and Hartatri (2010), the longer the period of shelf the rate of overhaul of food reserves and the use of cocoa seed energy is greater so that the seeds lack energy and eventually the growth of seed stems diameter more slowly.

Table 4. Average stem diameter values of 60 HST due to the effect of interaction between moisture level and cocoa seed saving period.

Moisture Content	Periods Save Seeds				
	0	2 weeks	4 weeks	6 weeks	8 weeks
 Diameter of Seed Stems (mm)				
20-23%	35.70 bA (5.97)	25.10 bA (5.00)	3.00 aA (1.63)	3.20 aA (1.68)	0 aA (0.71)
25-28%	29.80 bA (5.46)	32.70 bA (5.72)	26.10 bB (5.11)	17.70 bB (4.03)	0 aA (0.71)
30-33%	25.80 bA (5.08)	21.80 bA (4.67)	3.30 aA (1.70)	0 aA (0.71)	0 aA (0.71)
35-38%	25.70 bA (5.07)	26.60 bA (5.16)	14.60 bB (3.71)	3.20 aA (1.68)	0 aA (0.71)
BNJ 0.05			1.68		

Description: The numbers followed by the same letter (lower case in the same row, capital letters in the same column) show no significant difference at the 5% probability level; data transformation.

The result of the analysis showed that the interaction treatment of moisture level and the shelf period affected the dry weight of normal seedlings, but did not show any significant difference in each treatment (Table 5). Dry weight of the most heaviest normal seed is found in the interaction of 20-23% moisture content and without storage process, at the same interaction also with the increase of moisture content of dry weight

of seedlings become decreasing and decreasing with increasing seed saving period. Lodong *et al.* (2015) states that the dry weight of seedlings is strongly associated with germination where the higher the germination rate, the growth of the seedlings will be faster, thus resulting in heavier dry weight. So if the germination rate is relatively the same, it will give the same effect to the dry weight of the seedlings.

Table 5. Average dry weight values of 60 HST normal seedlings due to the influence of interaction between moisture level and cocoa seed saving period.

Moisture Content	Periods Save Seeds				
	0	2 weeks	4 weeks	6 weeks	8 weeks
 Dry Weight Normal Seeds (gr)				
20-23%	6.22 bA (2.49)	4.07 bA (2.02)	0.44 aA (0.77)	0.29 aA (0.67)	0 aA (0.71)
25-28%	6.06 cA (2.46)	4.87 cA (2.21)	3.71 bcB (1.91)	2.81 bB (1.62)	0 aA (0.71)
30-33%	6.01 bA	5.21 bA	0.45 aA	0 aA	0 aA

Moisture Content	Periods Save Seeds				
	0	2 weeks	4 weeks	6 weeks	8 weeks
 Dry Weight Normal Seeds (gr)				
	(2.45)	(2.28)	(0.78)	(0.71)	(0.71)
35-38%	5.55 cA	4.30 cA	2.42 bB	0.30 aA	0 aA
BNJ 0.05	(2.35)	(2.07)	(1.50)	(0.68)	(0.71)
			0.55		

Description: The numbers followed by the same letter (lower case in the same row, capital letters in the same column) show no significant difference at the 5% probability level; data transformation.

Good plant growth can be characterized by high dry weight and is influenced by the rapidity of roots reaching nutrients in the soil, increasing the number and length of plant roots (Nainggolan, 2001). Seeds with high vigor can form and transmit raw materials to the embryo shaft rapidly thus increasing the accumulation of dry matter. The high dry weight can illustrate the efficient utilization of food stocks in seeds (Nurussintani *et al.*, 2012). According to Ardian (2008), the weight of sprouts is influenced by the length of growth from the beginning until the process of germination, because if the sprouts take a long time to grow then the sprouts obtained are short sprouts, small sprouts, short hypocotyl, and small root volume. The dry weight of sprouts is one indicator of seed vigor, the high value of dry weight of sprouts indicates the high vigor of seed (Justice and Bass, 2002). Good plant growth can be characterized by

high dry weight and is influenced by the rapidity of roots reaching nutrients in the soil, increasing the number and length of plant roots (Nainggolan, 2001).

Seeds with high vigor can form and transmit raw materials to the embryo shaft rapidly thus increasing the accumulation of dry matter. The high dry weight can illustrate the efficient utilization of food stocks in seeds (Nurussintani *et al.*, 2012).

The Influence of Water Level and Time Period of Save on Electric Conductivity

Electrical conductivity is a physical seed test that reflects cell membrane leakage levels. The result of variance on electrical conductivity parameters shows that the treatment of water content has significant effect, while the treatment of the shelf period is very significant.

Table 6. Average electrical conductivity values due to the influence of moisture level and cocoa seed saving period.

Treatment	Electrical Conductivity (ppm)
Moisture level	
Moisture content 20-23%	0.17 a (0.81)
Moisture content 25-28%	0.13 a (0.79)
Moisture content 30-33%	0.16 a (0.81)
Moisture content 35-38%	0.13 a (0.79)
BNJ 0.05	0.06
Period save	
without save	0.03 a (0.73)
2 weeks saving period	0.08 ab (0.76)
4 weeks saving period	0.22 b (0.85)
6 weeks saving period	0.21 b (0.84)
8 weeks saving period	0.19 b (0.83)
BNJ 0.05	0.07

Description: The number followed by the same letter in the same column is not significant at the 5% probability level; data transformation.

The result of statistical test in Table 6 shows that the value of conductivity of the water treatment level did not show any significant difference, the average value of the electric conductivity that is produced tends to be the same each level of water content. The storage period treatment showed that without storage process, the average electrical conductivity yield was 0.03 ppm, when the seeds were stored for 2 weeks the resulting electrical conductivity average increased 0.08 ppm and if the seeds were stored for 4 weeks then the resulting DHL increased to 0.22 ppm. The highest mean electrical conductivity value was found in seed saving period for 4 weeks 0.22 ppm but not significantly different with seed saving period for 2, 6, and 8 weeks.

According to Rahayu *et al.* (2014) the value of electrical conductivity for cocoa seeds tends to increase along with decreasing of seed water content. The conductivity of cocoa seed immersion continues to increase with the increase in the time of seed storage. Decrease in water content causes leakage of membrane and cacao seed cell wall increased as indicated by increased electrical conductivity, leakage P, and leakage K. The lower the seed water level, the higher the membrane leakage value and the cell wall is inversely proportional to its viability.

Rahardjo (2012) states that the increase in electrical conductivity of soaking water shows that there has been a change in the permeability of the cell membrane of the seed so that more chemicals are released from the cell due to the auto-oxidation process of free radical groups. The more chemical compounds that come out of the cells into the seed water will increase the value of electrical conductivity.

The value of the correlation coefficient (0.89) indicates that the electrical conductivity is closely related to the length of the shelf period, clearly the curve of electrical conductivity values based on the length of cocoa seed storage can be seen in Fig. 1. The picture shows that the longer the seed saving period, the higher the value electric conductivity generated. The mathematical calculation results from the quadratic regression model has obtained the

maximum electrical conductivity value achieved in the 6th week saving period and at the 8th week saw a decrease in the return value of electrical conductivity. The relationship explains that as the length of saving period of a seed the value of its electrical conductivity continues to increase until it reaches a certain point and after passing through the peak of the decline.

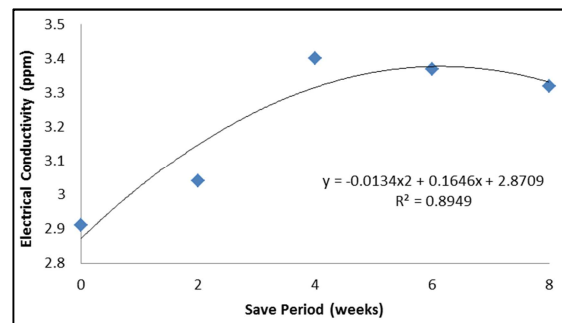


Fig. 1. Graph of electrical conductivity relation with period of shelf of cocoa seed.

Rohandi and Widyani (2016) found the same thing in research on *Shorea stenoptera* seeds that biochemical indication can also be seen from the value of electric power of *Shorea stenoptera* seed which is getting bigger with the longer time of seed storage. The value of electrical conductivity will increase with the decrease of seed vigor so that the seed will tend to leak the metabolite materials it contains and the leakage in the cell membrane is a place of significant damage from the decline of seed (Manju and Kumar, 2015). The dissolved nature of the solute contains many potassium, sugars, and amino acids that contribute to seed germination. During the imbibition process there is loss of solute from seed due to membrane construction disruption or cell damage which greatly reduces the seed germination potential to grow into healthy seedlings. Therefore, conductivity can be used as one of the seed viability indicators (Ramos *et al.*, 2012).

Effect of Water Levels and Old Period Save on Free Fatty Acids

The result of the analysis of variance showed that the treatment of water level had significant effect on free fatty acid, while the shelf period treatment had a very significant effect on cocoa seed free fatty acid.

Table 7 shows that fatty acid content tends to undergo changes in composition as well as ups and downs in both the water content and the cocoa seed storage period. Water level treatment did not show significant difference to free fatty acid value, but in line with

decreasing of moisture content, the value of free fatty acid decreased. While the treatment of the shelf period showed that the highest free fatty acid values were encountered in 4 week saving period significantly different from other storage periods.

Table 7. Mean value of free fatty acid due to the influence of moisture level and cocoa seed saving period.

Treatment	Free Fatty Acid (%)
Moisture Level	
Moisture content 20-23%	1.73 a (7.17)
Moisture content 25-28%	1.64 a (7.06)
Moisture content 30-33%	2.14 a (7.96)
Moisture content 35-38%	2.06 a (7.93)
BNJ 0.05	1.85
Period save	
without save	0.88 a (5.39)
2 weeks saving period	0.78 a (5.07)
4 weeks saving period	3.80 c (11.15)
6 weeks saving period	1.82 b (7.62)
8 weeks saving period	2.16 b (8.43)
BNJ 0.05	2.03

Description: The number followed by the same letter in the same column is not significant at the 5% probability level; data transformation.

Damage to seed during storage and declining seed quality depends on the relative temperature and humidity of storage space, storage time, and initial moisture content of the seed. Increased seed water levels lead to increased enzyme activity, such as hydrolysis enzymes. Enzymes already exist in the seeds and will be active when the seeds absorb water. Activity is increasing when more water availability. One of the hydrolysis enzymes is lipase that hydrolyzes fat to fatty acids and glycerol (Wolfram and Spener, 2001), and increases in free fatty acid content via seed viability decreases. Whitefield (2005) states that cocoa seed-free fatty acids are increased in stored seeds with high water content due to increased lipase activity.

The relationship between the old cocoa seed shelf period and the free fatty acid value can be seen in Fig. 2. The quadratic coefficient value (0.4605) shows that

the diversity of free fatty acid value is 46.05%. The maximum free fatty acid value is seen at week 5 and continues to decline until the 8th week.

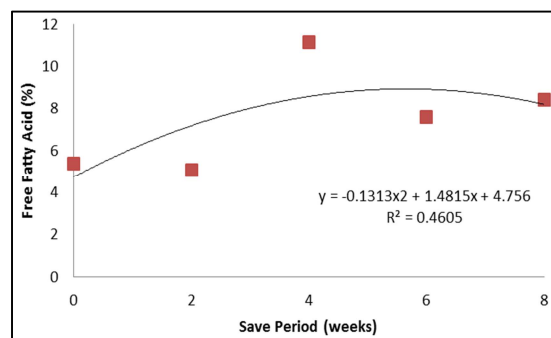


Fig. 1. Graph of free fatty acid relationship with cocoa seed saving period.

Biochemical deterioration symptoms in seeds are changes in enzyme activity, respiration rate, increased fatty acid, and reduced supply of food reserves. The high fatty acid content in the seeds is an indication of

fatty acid accumulation, since it is not processed further into energy so that the seed loses energy to germinate (Tresniawati *et al.*, 2014).

Conclusion

Water level affects the viability and vigor of cocoa seeds. The decrease in viability and vigor of the seeds is closely related to the moisture content of the seed. Storage of cocoa seeds at moisture content of 35-38% can suppress the rate of decline of seed. The length of the shelf period affects the viability and vigor of cocoa seeds. The longer the seed is stored, the lower the viability and the vigor. Viability and vigor of seeds even in the 6 week saving period have already shown seed death. There is an interaction between the moisture level and the length of the shelf period on the viability and vigor of cocoa seed. The rate of decline of cocoa seeds in each saving period depends on the degree of moisture content of the seed. Up to the 6-week saving period, viability and vigor of cocoa seeds can be maintained at a 25-28% moisture level.

References

- Adelina and Maemunah E.** 2004. Cutting and Giving Cytokinins at Cocoa Bean Root. *Journal Agroland* **11(3)**, 255-260.
- Ardian.** 2008. Effect of Treatment of Temperature and Heating Time of Seeds to Arabica Coffee Growth (*Coffea arabica*). *Deed of Agrosia* **11(1)**, 25-33.
- Directorate General Plantation.** 2013. Area of Plantation Area in Indonesia 2008-2012. Ministry of Agriculture Republic Indonesia, Jakarta.
- Esrita.** 2009. Anatomy Study of Embryo of Cocoa Seed on Several Seed Water Content and Drying Level. *Journal of Agronomy* **13(1)**, 1-5.
- Justice OL, Bass LN.** 2002. Seed saving Principles and Practices. PT Raja Grafindo, Jakarta.
- Khrisnan P, Nagarajan S, Moharir AV.** 2005. Biophysical Characteristic of Seed Water Status and Its Relationship with Seed Water Status. *Proc. Indian Natl. Sci. Acad* **71(3)**, 163-179.
- Lodong O, Tambing and Adrianton Y.** 2015. Role of Packaging and Store Media on Endurance and Vigor Seeds of Jackfruit (*Artocarpus heterophyllus* Lamk.) Tulob-5 Cultivars During Storage. *e-Journal Agrotekbis* **3(3)**, 303-315.
- Manju V, Kumar S.** 2015. Seed Leachate Conductivity and Its Correlation with Seed Viability and Germination of *Thau papaya* Cv. Co8 Seeds Stored Under Different Environmental Conditions. *International Journal of Agricultural Science and Research (IJASR)* **5(4)**, 127-130.
- Minister of Agriculture Regulation.** 2013. Standard Operating Procedures for Seed Garden Regulation, Seed Certification, and Evaluation of Cocoa Crop Seed Sources (*Theobroma cacao* L.). NUMBER 90/Permentan/OT.140/9/2013.
- Nainggolan T.** 2001. Response Oil Palm Seeds in Early Breeding to Organic Ingrediation of Kacscing and Inoculant CMA. *Journal Eksakta-Biagrotek* **1(1)**, 6-11.
- Nurussintani W, Damanhuri and Purnamaningsih SL.** 2012. Treatment of Dormancy Destruction on Seedling Power of 3 Peanut Varieties (*Arachis hypogaea*). *Journal of Plant Production* **1(1)**, 86-93.
- Prawoto A.** 2008. Plant Propagation Complete Cocoa Guide Agribusiness Management from Upstream to Downstream. The Swadaya spreader. Bogor Agricultural World Information 74-90.
- Purwanti S.** 2004. Study Room of temperature saved on quality of black soybean seed and yellow soybean. *Agricultural Science* **11(1)**, 22-31.
- Rahardjo P, Hartatri DFS.** 2010. Use of Acrylic Acid Sodium Polymer in Effort to Maintain the Viability of Cocoa Seed (*Theobroma cacao* L.). *Pelita Perkebunan*, **26(2)**, 83-93.
- Rahardjo P.** 2012. Influence Rice Ash Absorbing as Husk Material on Seed Storage to Growing Power and Cocoa Seed Growth. *Pelita Perkebunan* **28(2)**, 91-99.

Rahayu A, Hardiyati T, Hidayat P. 2014. Effect of Polyethylene Glycol 6000 and Long Storage of Quality of Cocoa Seed (*Theobroma cacao* L.). Pelita Perkebunan **30(1)**, 15-24.

Ramos KMO, Matos JMM, Martins RCC, Martins IS. 2012. Electrical Conductivity Testing as Applied to the Assessment of Freshly Collected *Kielmeyera coriacea* Mart. Seeds. International Scholarly Research Network (ISRN) Agronomy 1-5.

Rohandi A, Widyani N. 2016. Physiological and Biochemical Changes of Tengawang Seeds during Storage. Journal of Dipterocarppa Ecosystem Research **2(1)**, 9-20.

Sulaiman F, Harun MU, Kurniawan A. 2010. Germination of Rubber Seed Plants (*Hevea Brasiliensis* Muell. Arg.) Saved at Different Temperatures and Periods. Proceedings of the National Seminar, 13-14 December 2010: 1593-1603, Palembang.

Sumampow DMF. 2011. Viability of Cocoa Seed (*Theobroma cacao* L.) in Sawdust Media. Faculty of Agriculture, Sam Ratulangi Manado University. Soil Environment **8(3)**, 102-105.

Tambunsaribu DW, Anwar S, Lukiwati DR. 2017. Seed Viability and Growth of Cocoa Seedlings (*Theobroma cacao* L.) in Some Types of Store Media and Humidity Level. Journal Agro Complex **1(3)**, 135-142.

Tresniawati C, Murniati E, Widajati E. 2014. Physical, Physiological and Biochemical Changes Ripening Seed and Recruitment Study of Candlenut Sunan Seed. Indonesian Agronomy Journal **42(1)**, 74-79.

Whitefield R. 2005. Making Chocolates in the Factory Kennedys Publications Ltd., London.

Wolfram C, Spener F. 2001. Fatty Acids as Regulators of Lipid Metabolism. Eur. J. Lipid Sci. Technology **102**, 746-762.

Yuniarti N, Djaman DF. 2015. Proper Packing Techniques for Maintaining Vulnerable Seed Viability (*Rhizophora apiculata*) during Storage. Proceedings of the National Seminar of Indonesian Biodiversity Society **1(6)**, 1438-1441.