

The storage capacity of cocoa seeds (*Theobroma cacao* L.) through giving Polyethylene Glycol (PEG) in the various of storage container

R R Lahay, S Misrun and R Sipayung

Department of Agrotechnology, Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia

E-mail: ratna.rlahay@gmail.com

Abstract. Cocoa is plant which it's seed character is recalcitrant. Giving PEG and using various of storage containers was hoped to increase storage capacity of cocoa seeds as long as period of saving. The reseach was aimed to identify the storage capacity of cocoa seeds through giving PEG in the various of storage containers. Research took place in Hataram Jawa II, Kabupaten Simalungun, Propinsi Sumatera Utara, Indonesia. The method of this research is spit-split plot design with 3 replication. Storage period was put on main plot which was consisted of 4 level, PEG concentration was put on sub plot, consisted of 4 level and storage container was put on the sub sub plot consisted of 3 types. The results showed that until 4 days at storage with 45 % PEG concentration at all storage container, percentage of seed germination at storage can be decreased to be 2.90 %, and can be defensed until 16 days with 45 % PEG concentration at perforated plastic storage container. Percentage of molded seeds and seed moisture content were increased with added period of storage but seed moisture content was increased until 12 days at storage and was decreased at 16 days in storage.

1. Introduction

Recalcitrant seeds originated from tropical and subtropical plant species are characterized by the absence of maturation drying, and most of them have a high water content and active metabolism when they are shed from the mother plant. They are sensitive to drying and low temperatures, and quickly lose viability during storage [1,2]. Seeds of many important economical plants are recalcitrant, including many important tropical plantation crop species such as rubber and cocoa, tropical fruit crops such as mango, lychee and longan, and tropical timber species which belong to the families Dipterocarpaceae and Araucariaceae, etc. Seed germination during storage can be inhibited by using growth inhibitors such as Polyethylene glycol (PEG) which maintains the osmotic potential of cells that can be used to limit changes in water content and O₂ in germination or storage medium so that PEG molecules that are outside the seed cell membrane will forming a thin layer that protects the seeds and serves as a buffer of seed water and oxygen inlet [3]. Based on the description above, we interested to conduct research to know the provision of PEG-6000 to the cocoa seed with various storage container so that later beneficial in terms of delivery of seeds outside the area without any seeds germinated during the trips.



2. Materials and Methods

This research was conducted at farm in Jl. Hataran Jawa II Simpang Pure Kabupaten Simalungun, and Tissue Culture Laboratory of Faculty of Agriculture, University of Sumatera Utara, Medan, Indonesia.

2.1. Statistical analysis

This study uses Split Split Plot Design with three treatments. Main plot is storage period (T) with four levels : 4, 8, 12, and 16 days. Sub plot is concentration of PEG-6000 (P) with four levels : 0, 15, 30, and 45 %. Sub-sub plot is storage container (S) with three types : perforated plastic, plastic + sawdust, and plastic + hush ash. Data are presented as means- standard deviation of three replicates. The statistical differences were tested using an analysis of variance (ANOVA) and means were compared used Duncan Multiple Range - test. Figures show significant differences using letter designations. Data points with different letters show significant differences ($P < 0.05$).

2.2. Pulp cleansing and fungicidal seed treatment

The material used in this research is cocoa seed clone DR 38. Cocoa fruit is split with a batter, then taken seeds in the middle of the fruit, and cleaned the pulp of cocoa beans with ash. After clean pulp, washed with water and soaked for 5 minutes in a Delsene MX-80 WP fungicide solution with a concentration of 2 g / l of water then dried for 1 hour.

2.3. Treatment of PEG-6000 concentration

Seeds that have been soaked with fungicide are then incorporated into PEG-6000 (gr / vol) solution for 10 minutes according to the treatment concentration level and then dried for 5 hours.

2.4. Seed storage

The treated seeds were stored in the medium according to the storage container treatment, where the sawdust and the husk ash to be used as storage container, soaked with Delsene MX 200 fungicide then dried and then stored into room temperature for 4, 8, 12, 16 days.

2.5. Seed germination

Germination media is 10-15 cm thick layer sand. The seeds are sowed with radicles at the bottom with a seed spacing of 2 cm x 3 cm and a 1 cm of planting depth. Watering done twice a day ie in the morning and afternoon with handsprayer.

3. Results and Discussion

3.1. Percentage of seeds germinated in storage

Storage duration (T), PEG concentration (P), storage container (S), interaction of T x P; T x S; P x S and interaction of T x P x S significantly affect the percentage of seed germinated in storage. Data percentage of seeds germinated in storage are presented in Table 1.

The lowest of percentage seed germinated in storage (2.90%) was in the seed treatment with PEG 45% in perforated plastic containers for 12 days which was not significantly different with the treatment of seeds with PEG 45% in 4 or 8 day in perforated plastic containers as well were not significantly different from seed treatment at all PEG concentrations (0, 15, 30, 45%) on plastic containers + sawdust and plastic + ash husk stored for 4 days. For each use of storage container, it is increasing PEG concentrations at storage time of 4, 8 and 12 days will prevent the percentage of seeds germinated during storage.

PEG in some concentrations has demonstrated its role in suppressing water absorption. This suggests that the higher concentrations of PEG used may decrease the number of seeds germinated during storage although it can not prevent seed germinate by 0 %. PEG has certain osmotic values equal to the osmotic value of cocoa seed, is expected to prevent imbibition so that the seed germination process will not occur because the imbibition is the main condition of germination [3].

Table 1. Percentage of seeds germinated in storage in the interaction of storage period, PEG concentration and storage container

Storage period	PEG Concentration	Storage container		
		Perforated plastic	Plastic + sawdust	Plastic + husk ash
	 %		
4 days	0 %	49.63 ef	5.42 j	13.49 j
	15 %	37.07 g	5.42 j	6.93 j
	30 %	26.50 hi	2.90 j	6.93 j
	45%	2.90 j	2.90 j	2.90 j
8 days	0 %	73.18 ab	46.26 f	84.58 a
	15 %	68.31 bc	37.77 g	80.55 a
	30 %	53.72 def	36.11 g	79.39 ab
	45%	10.60 j	20.42 hij	65.60 bc
12 days	0 %	57.66 cde	83.05 a	82.03 a
	15 %	26.57 h	60.77 cd	87.13 a
	30 %	7.94 j	49.58 ef	81.94 a
	45%	2.90 j	48.83 ef	79.39 ab
16 days	0 %	14.11 ij	2.90 j	7.94 j
	15 %	6.93 j	2.90 j	2.90 j
	30 %	2.90 j	2.90 j	2.90 j
	45%	2.90 j	5.42 j	2.90 j

3.2. Percentage of moldy seeds in storage

Storage period (T), storage container (S), interaction of T x S; P x S had a significant effect on the percentage of moldy seeds in storage, but PEG concentration (P), interaction T x P; and interaction of T x P x S had no significant effect on percentage of moldy seeds in storage. Data percentage of moldy seeds in storage are presented in Table 2 and Table 3.

Table 2. Percentage of moldy seeds in storage in the interaction of storage period with storage container

Storage Period	Storage container		
	Perforated plastic	Plastic + sawdust	Plastic + husk ash
 %		
4 days	2.90 c	2.90 c	2.90 c
8 days	3.53 c	2.90 c	2.90 c
12 days	13.14 b	8.06 c	4.54 c
16 days	24.70 a	15.50 b	4.79 c

The longer of storage period for all types of storage container, the percentage of moldy seeds in storage is increasing. The lowest percentage of moldy seeds (2.90%) was obtained at seed storage up to 8 days in plastic container + sawdust or plastic container + husk ash which was not significantly different with 4 days seed storage in each type of storage container (perforated plastic, plastic + sawdust, and plastic + ash husk), also not significantly different with 12 day storage using plastic + sawdust or plastic + husk ash and 16 day storage using plastic + husk ash.

High levels moisture content of recalcitrant seed during storage have several risks, namely seed germination and growth of fungus in storage. According to [4] stated that cocoa seeds including recalcitrant seeds have the unstable properties stored at moisture content of less than 12% and at

temperatures. Too low, while the fungus can grow and develop well at high seed moisture content and temperature levels so that cocoa seeds are susceptible to fungal attack during storage. The longer the seeds are stored, the fungus will continue to grow so as to decrease the power of seed sprouts.

Table 3. Percentage of moldy seeds in storage (%) in the interaction of PEG concentration with storage container

PEG Concentration	Storage container		
	Perforated plastic	Plastic + sawdust	Plastic + husk ash
%		
0 %	7.72 bcd	4.66 cd	4.16 d
15 %	9.90 abc	12.12 ab	2.90 d
30 %	12.89 ab	6.46 cd	4.16 d
45 %	13.76 a	6.12 cd	3.91 d

In the use of perforated plastic containers, increasing PEG concentration will increase the percentage of moldy seeds. When using a storage container in the form of plastic + husk ash, then the increase of PEG concentration in the seeds has no significant effect on the percentage of moldy seeds. The lowest percentage of moldy seeds (2.90%) was found in seed treatment with 15% PEG stored in plastic + husk ash. It can be seen that perforated plastic container is unable to suppress the growth of the fungus during storage because by giving the aeration hole, oxygen and free air out and into the seed so that the fungus can be carried by air and attached to the seed and spread to the other seed. In the plastic + husk ash container, the percentage of moldy seeds in storage is relatively low when compared with other storage containers. This is because in the container store the ash husk has passed through the combustion process so that the pathogen microbes have died during the burning process.

3.3. Seed moisture content after storage

Storage period (T), PEG concentration (P), storage container (S), interaction of T x P; T x S had significant effect on seed moisture content, and interaction of P x S, and interaction of T x P x S not significant effect on seed moisture content. Data seed moisture content after storage are presented in Table 4.

Table 4. Seed moisture content in the interaction of storage treatment with storage container

Storage Period	Storage container		
	Perforated plastic	Plastic + sawdust	Plastic + husk ash
%		
4 days	45.31 bc	35.88 e	38.39 d
8 days	43.80 c	43.52 c	47.76 b
12 days	33.11 f	45.85 bc	53.30 a
16 days	25.91 g	12.12 h	24.25 g

When using perforated plastic storage container, the longer the seeds are stored, the moisture content of the seeds decreases. But when using a storage container in the form of plastic + sawdust and plastic container + husk ash, then the water content of the seeds will increase until the storage time of 12 days, then the water seed will decrease after the seeds are stored 16 days. The highest seed water content (53.30%) was found in seeds stored in plastic + husk ash for 12 days which was significantly different from other treatment combinations.

According [5] that the high moisture content of seed during storage due to additional water from the process of respiration seed. Increased moisture content of this seed is in line with the reduced carbohydrate content in the seeds. The reducing sugar content is closely related to the respiration rate of the seeds in which the higher the sugar content of the respiration rate of the seed respiration rate

will increase. As a result the water content in the seeds will increase because in the process of respiration will be released water. Therefore seeds stored for 12 days have high water content but will experience decrease when stored longer due to decline of seed viability. Recalcitrant seeds can not stand stored for long periods of time and there will be a process of declining the seed during storage.

Storage containers affect changes in moisture content after storage. In perforated plastic container, oxygen (O₂) and other gases can come out and enter into the container that causes the seed to do the imbibition. Sawdust has a hygroscopic property so that the water vapor attached to sawdust will be absorbed by the seed so that the determination of the container is very crucial since the cocoa seed is the recalcitrant seed.

3.4. Percentage of seeds germination in nursery

Storage period (T), storage container (S), interaction of T x S, significantly effect on percentage of seed germination, while PEG concentration (P), interaction of T x P; P x S, an interaction of T x P x S had not significant effect on the percentage of seed germination in nursery. Data percentage of seeds germination in nursery are presented in Table 5.

Table 5. Percentage of seeds germination in nursery in the interaction of storage period with storage container

Storage Period	Storage container		
	Perforated plastic	Plastic + sawdust	Plastic + husk ash
 %		
4 days	84.96 a	86.41 a	84.96 a
8 days	87.13 a	87.13 a	84.56 b
12 days	87.13 a	87.13 a	87.13 a
16 days	85.28 a	7.22 c	81.67 b

The seed storage up to 12 days with different containers resulted in a high percentage of seeds germination in nursery. Especially when using perforated plastic containers, storage up to 16 days can still maintain the percentage of seeds germinated in the nursery is high enough. Meanwhile, when using a plastic + sawdust and plastic + husk ash stored up to 16 days will decrease percentage of seeds germination in nursery.

Storage containers have a great influence on seed conditions during storage. Environmental factors such as water and oxygen have an effect on seed germination. The moisture content of the seeds is too low causing decreased power growing seeds and cocoa seeds belonging to recalcitrant seeds are not able to withstand excessive dehydration and will soon lose its viability at relatively low humidity. According to [6] states that in general the recalcitrant seeds are high moisture content and the moisture content is correlated with its viability.

4. Conclusion

Treatment of cocoa seeds with PEG 45% stored in perforated plastic container up to 12 days can minimize the percentage of seeds germinated during storage up to 2.90%. The longer of storage period for all types of storage container, the percentage of moldy seeds in storage is increasing. The lowest percentage of moldy seeds (2.90%) was found in seed treatment with 15% PEG stored in plastic + husk ash. The highest seed water content (53.30%) was found in seeds stored in plastic + husk ash for 12 days. The seed storage up to 12 days using different storage containers can still maintain a high percentage of germination seeds in nursery.

References

- [1] Roberts, E H 1973 Predicting the storage life of seeds Seed Sci. Technol. **1** pp. 499 - 514.

- [2] Vertucci C W and Farrant J M 1995 Acquisition and loss of desiccation tolerance In Kigel J, Galili G (Eds.) *Seed Development and Germination*. Marcel Dekker Inc. New York. pp. 237- 271
- [3] Rahardjo P 1986 Penggunaan Polyethylene Glikol (PEG) sebagai medium penyimpanan benih kakao (*Theobroma cacao* L.) *Pelita Perkebunan* **II** (3) pp. 103 – 108
- [4] Pusat Penelitian Kopi dan Kakao Indonesia 2004 *Panduan lengkap budidaya kakao* Agromedia Pustaka Jakarta
- [5] Purwaningsih O 2001 Kajian fisiologis dan biokimiawi benih rambutan (*Nephelium lappaceum* L.) selama penyimpanan dengan perlakuan ABA dan GA₃. *Ilmu Pertanian* Vol. **8** (2) pp. 66-75
- [6] Sutopo L 1985 *Teknologi Benih (Seed Techonology)* Rajawali Jakarta