Germination seed of cacao (*Theobroma cacao* L.) on some osmotic solutions

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Abstract. Cocoa seeds are classified as recalcitrant seeds that quickly lose their growing power after being harvested and stored. This relates to the procurement of seeds for plantations whose location is quite far from the source of the seed. This study aims to determine the effect of some osmotic solutions on cocoa seed germination that has undergone storage. The study was conducted at the Seed Technology Laboratory of the Faculty of Agriculture, University of North Sumatra using a randomized block design with 2 treatment factors. The first factor was osmotic solution, namely: control (without osmotic solution), distilled water, 50% coconut water, 100% coconut water, 5% PEG and 10% PEG. The second factor is the storage time at room temperature, namely: control (0 days), 2 days, 4 days and 6 days. The results of this research showed that the osmotic solution used significantly improved the germination of cocoa seeds as indicated by the increase the vigor index. The osmotic solution of coconut water significantly increases the vigor index of seeds that have been stored for 2 days from 3.61 to 5.75.

1. Introduction

Cocoa plants (*Theobroma cacao* L.) come from tropical rainforests in Central America and northern South America. This plant was introduced by the Spaniards in 1560 in Minahasa, Sulawesi [1].

Indonesia is one of the largest cocoa cultivators in the world and is the third largest cocoa producing country after Ivory-Coast and Ghana. According to plantation statistics, the area of cocoa plantations in Indonesia is 1,722,315 ha with a national production of 760,430 tons in 2016. This production has increased compared to 2015 production, which amounted to 661,243 tons [2]. In the North Sumatra region alone, cocoa production also increased from 133,853 tons in 2015 to 152,813 tons in 2016 [3]. Most of cocoa plantations (almost 95%) are managed by the communities and only a small portion are managed by large state plantations and large private plantations [4]. Thus the cocoa business development will directly or indirectly affect the people's economy.

Cocoa planting material is the basic capital to achieve high cocoa production. Errors in the selection and use of planting material will result in long-term losses. One of the obstacles in the development of cocoa to areas of development centres is the availability of quality seeds from superior varieties and the very limited shelf life of seeds and cocoa enters [5]. Cocoa beans are recalcitrant seeds that quickly lose their germination power after being removed from the fruit. But if it is still stored in the fruit, the seeds can also be attacked by disease and can reduce its viability after 15 days of storage [6]. Delivery of cocoa seeds in the fruit will also increase transportation costs.

Farmers usually remove cocoa beans from their fruit and leave them on a stretch in a few days when preparing the planting area. This will result in a decrease in the water content in the seeds. According to the results of the study, the decrease in water content of cocoa seeds up to 21-23% has not reduced its potential viability, but the vigor has decreased in the water content of about 25%. But at 14-15% moisture content the viability is very low [7]. To increase viability of the seeds which have deterioration, often done with several methods invigoration. Example use PEG 6000 in soybeans [8], kenaf [9] and there are also organic materials such as coconut water on red chili [10].

Coconut water contains several growth hormones that can stimulate plant growth. Hormones contained in coconut water are cytokinin (5.8 mg / l), auxin (0.07 mg / l) and a little giberelin and other compounds that can stimulate germination and growth [11].

Polyethylene glycol (PEG) is a compound that can reduce the osmotic potential of solutions through matrix activity of ethylene oxide sub-units capable of binding hydrogen water molecules [12]. It is not known exactly how long the storage time in room temperature can still be tolerated by the cocoa seeds after being removed from the fruit and is there any type of osmotic solution that can improve the quality of seeds that have experienced a decline for sowing in the field. For this reason this study was conducted.

2. Materials and Methods

The study was conducted at the Seed Technology Laboratory of the Faculty of Agriculture, Universitas Sumatera Utara with elevation ± 32 m above sea level. The ingredients used are cocoa fruit, coconut water, Polyethylene Glycol (PEG), distilled water (aquades), rubbing ash and sand. The equipment used is tub germination, Erlenmeyer, knife, and hand sprayer.

Seed preparation: cacao fruit is split and the seeds are removed. Then the seeds are cleaned from the pulp using rubbing ash until clean. These clean seeds are stored at room temperature according to treatment. Room temperature 20-31°C and relative humidity ± 64%. After storage, the seeds is followed by the treatment with osmotic solutions.

The study used factorial randomized block design with 3 replications and 2 factors. The first factor is storage time at room temperature which consists of: 0 days (D0), 2 days (D1), 4 days (D2) and 6 days (D3). The second factor is osmotic solution, consisting of: Control (S0), distilled water (S1), 50% coconut water (S2), 100% coconut water (S3), 5% PEG (S4) and 10% PEG (S5). Each combination of treatment uses 20 cocoa seeds. The total seed used is 1440 seeds. The results of the research which have a significant effect were continued with a different test of the mean using Duncan's multiple distance test with a level of 5%. The parameters observed were germination potential, germination test (normal, abnormal and dead germination), and vigor index.

3. Results and Discussion

The results of variance showed that the treatment of storage time at room temperature significantly affected all the parameters observed while the treatment of osmotic solution given did not significantly affect to the parameters observed. However, the interaction between the two treatments significantly affected to vigor index.

The potential seed germination and germination test of cocoa in the treatment of several osmotic solutions and storage time is shown in Table 1. Potential germination and normal percentages of germination showed decreases significantly with increasing seed storage time. In contrast, abnormal germination and dead seed percentage increased significantly (Table 1). This is because cocoa seeds include recalcitrant seeds that have a highwater content and cannot be stored for long time [13]. The longer the storage of seeds at room temperature will result in the seeds losing their liquid. The initial moisture content of cacao seeds is 35.6%. After being stored for 2, 4 and 6 days the seed moisture content was 16.9%, 8.8% and 6.0%. According to the results of the research at high water content, full metabolism can occur. At intermediate water some limited metabolism is possible, but is unregulated and can lead to oxidative damage. At lower water content is intimately associated macromolecular structures and its removal leads to direct damage to these structures [14]. The rapid decline in cacao

below the critical moisture level was correlated with remarkable increases in cell leakage and lipid peroxidation [15].

Table 1. The potential, normal, abnormal and dead germination of cacao in several osmotic solutions after storage.

Treatment	Potential germination (%)	Normal germination (%)	Abnormal germination (%)	Dead germination (%)
Storage time (days)				
0 (D0)	100.00 a	95.00 a	5.00 b	0.00 c
2 (D1)	99.44 a	93.06 a	6.39 b	0.56 c
4 (D2)	26.11 b	8.33 b	17.78 a	73.89 b
6 (D3)	0.28 c	0.28 c	0.00 c	99.72 a
Osmotic solution				
Control (S0)	58.75	46.25	12.50	41.25
Distilled water (S1)	58.33	51.67	6.67	41.67
50% Coconut water (S2)	57.08	48.75	8.33	42.92
100% Coconut water (S3)	57.08	51.67	5.42	42.92
5% PEG (S4)	55.00	48.33	6.67	45.00
10% PEG (S5)	52.50	48.33	4.17	47.50

Note: The numbers followed by different letters in the same column and treatment group are significantly different according to Duncan's test at the 5% level

A decrease in seed water content that is high enough will cause drying in the embryo so it suppresses ribosome activity in synthesizing proteins. Water levels that are too low will cause damage to the sub-cellular components, namely changes in enzyme structure, protein structure, and decreased cell membrane integrity [16]. Membrane damage that occurs during drying results in the release of hydrolytic enzymes during water absorption. Therefore excessive drying can accelerate the decline in seed viability [17]. The viability of cacao seeds has decreased significantly during the 4 days of drying (D2), which decreased from 93.06 to 8.33%. After 6 days of drying (D3), the percentage of dead seeds has reached 99.72%.

The results of research on *Medhuca indica* seeds which mature at 53% moisture content have an initial viability of 100%, if they experience natural drying until the water content is 39.4%, they will not suffer losses from germination. But the seeds are no longer viable if they are dried below 9.4% [18].

The treatment of osmotic solutions did not significantly affect the potential of germination, percentage of germination, abnormal germination and dead seeds. However, there is a tendency for osmotic solutions to suppress abnormal germination but increase dead seed compared to control or distilled water treatment. This is probably due to the presence of stimulants in coconut water such as giberalin. The content of gibberellins in coconut water stimulates cell growth and elongation in the sub-apical region of the meristem. The effect of gibberellins is to stimulate shoot lengthening, inhibit root growth, break seed dormancy so that it accelerates germination in plants [19]. The soaking female palm seeds in coconut water can increase the growth of female palm seedlings. Growth of palm seedlings begins to increase in the use of water with a concentration of 50% [20].

Vigor index of cocoa seed in the treatment of several osmotic solutions and storage time is shown in Table 2. The vigor index experienced a significant increase in cocoa seed storage for 2 days using

osmotic solution of coconut water and PEG. An increase in the vigor index also occurs in seeds that do not experience storage. The given osmotic solution cannot increase the index vigor of cocoa that has been stored for 4-6 days.

Table 2. Vigor index of cacao in several osmotic solutions after storage.

Osmotic solution	Storage time (days)				Average
	0 (D0)	2 (D1)	4 (D2)	6 (D3)	
Control (S0)	3.43 f	3.61 ef	0.80 g	0.00 g	1.96 b
Distilled water (S1)	6.05 abc	4.17 def	0.94 g	0.00 g	2.79 a
50% Coconut water (S2)	6.88 a	5.47 bcd	0.79 g	0.00 g	3.29 a
100% Coconut water (S3)	6.01 abc	5.75 abc	0.70 g	0.07 g	3.13 a
5% PEG (S4)	5.40 bcd	5.02 cde	0.52 g	0.00 g	2.74 a
10% PEG (S5)	6.32 ab	5.12 bcd	0.33 g	0.00 g	2.94 a
Average	5.68 a	4.86 b	0.68 c	0.01 d	

Note: The numbers followed by different letters in treatment group are significantly different according to Duncan's test at the 5% level

The cacao seed vigor index in coconut water treatment was higher than PEG treatment. This is because coconut milk contains several natural growth regulators such as giberalin and cytokinin and other nutrients, as stated by other researchers [11]. While PEG can only regulate the availability of water to the seed, but cannot enter into cells of the seed due to its high molecular weight. In their study, PEG priming improved germination energy and germination index and reduced mean germination time (MGT) as compared with the control, but PEG priming had longer MGT and lower germination index than salt priming [22].

4. Conclusions

Storage time of the cacao seed decreased significantly all parameters but osmotic solution only increased the vigor index. The osmotic solution of coconut water significantly increased the vigor index of the seeds that have been storage for 2 days from 3.61 to 5.75. For seeds no storage, increased from 3.43 to 6.88.

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