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PEG 6000 Ability Test and Fungicide Efficacy in Improving Storability of Shelled Rubber (*Hevea brasilliensis* Muell.Arg) Seed

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ABSTRACT
Rubber seeds are recalcitrant seed that are susceptible to fungal attack during storage, therefore it will lose strorability in a short time. The purpose of this study was to test the ability of polyethylene glycol 6000 (PEG 6000) to maintain strorability of shelled rubber seeds and the efficacy of fungicide to reduce fungal attack during storage. Completely randomized design was applied with two factors and three replications, i.e., PEG 6000 (%w/v): 0, 15, 30, 45, 60% and fungicide (active ingredients were Pyraclostrobin+Metiram): g/1 kg of seeds): 0, 10, 20, 30 and 40 g. The results showed that 30% PEG maintained strorability of 12 and 16 days where the fungus attacks were 18 and 42%, respectively. Fungicide of 40 g/1 kg was effectively inhibited the growth of fungal during storage, where at 12 and 16 days had fungal attack of 19 and 40%, respectively. The combination of PEG-6000 30% and fungicide of 40 g/1 kg was effective in improving the seed viability, the seeds were able to stand fungal attack during storage of 16 days to 18 with 96% germination. PEG 6000 and fungicide blended with the right combination as a media store, had significant effect in maintaining seed viability.

Key words: Rubber seed, recalcitrant seed, viability, storability, fungicide, polyethylene glycol 6000

INTRODUCTION
Handling rubber seeds for rootstock will determine the growth and production of rubber. Therefore rootstock seeds must be selected and managed appropriately (Ahmad and Raharjo, 2010). Rubber seeds are classified as recalcitrant that are susceptible to deterioration, not having dormancy time with high water content and will lose their germination in a short time (Pammenter and Berjak, 2000; Tian *et al*., 2004; Papadakis *et al*., 2005; Dias *et al*., 2010). According to Sembawa Research Institute (2009), when rubber seeds were exposed to sunlight for 7 days germination decreased from 80-0%. If no direct sunlight exposer the germination will decrease to 0% after 14 days. During the storage period, the seed should not be stored in jute sacks because it will hot and fermented so that germination will decrease. During conventional storage, seeds were mixed with moist sawdust, (1:1) in jute sacks and packed in wood containers. The procedure was expensive due to the addition of an exceptional delivery volume (Sembawa Research Institute, 2009). The use of sawdust as a storage medium has been conducted

by Zanzibar and Mokodompit (2007) with no satisfactory results, especially in soft-skinned mahogany seeds (recalcitrant) that cause negative effect on germination. The sawdust material used was relatively large (<600 μm), it would absorb and release the water so that the treatment was similar to soaking of seeds for several days in unfavorable conditions; the atmosphere around the seed became more moist and also triggered the growth of fungi. The use of sawdust was more appropriately applied to the impermeable seeds. In order to increase the selectivity of the seed to water and air, the researchers extracted the shell of rubber seeds. As a replacement of the shells, seeds were coated with polyethylene glycol 6000 (seed coating) as a compounds which have osmotic potential that could limit the availability of water and oxygen in the storage medium (Krizek, 1985; Nio *et al.*, 2011); osmotic potential in PEG 6000 had also been used by Lian *et al.* (2004) that PEG 6000 might play a role in avoiding simulated drought on rice plants. PEG ($HO-CH_2-(CH_2-O-CH_2)$ $x-CH_2-OH$) is a long-chain polymer compounds, inert, non ionic and non-toxic (Michel and Kaufmann, 1973). The most commonly used in research in plant and seed physiology were PEG 4000 and PEG 6000 (Krizek, 1985). Sumayku (2002) was successfully inhibited the germination of seeds using PEG 6000 in the conservation of mango seed. Arumingtyas *et al.* (2012) could solve drought stress in soybean germination phase. The purposes of this research was to determine the abihty of PEG 6000 to maintain shelf life of shelled rubber seed and efficacy of fungicides to reduce fungal attack during storage and maintaining the seed viability.

MATERIALS AND METHODS

Research was conducted at seed technology laboratory Agronomy Department, Faculty of Agriculture, Sumatera Utara University, Medan, Indonesia from May to August 2010. The materials used were PB 260 rubber seed (moisture content, 39.65%), polyethylene glycol 6000 (PEG-6000), a fungicide with active ingredient (a.i) of pyraclostrobin 55 g/1 kg and metiram 550 g/1 kg (Cabrio Top 60 WP). Insecticide used was Sevin 80S. Completely Randomized Design (CRD) was used this experiment with two factors in three replications. The first factor was PEG 6000 (%w/v): 0, 15, 30, 45 and 60%. The second factor was fungicide with a.i pyraclostrobin and metiram (g/1 kg of seeds): 0, 10, 20, 30 and 40 g. Data were analyzed by analysis of variance followed by Duncan Mean Range Test (DMRT) at $\alpha = 5\%$. During the storage period fungal seed (%) and germination (%) were observed on day 4, 8, 12 and 16. Equipments used were: oven, shell seed breaker, hand sprayer, measuring cups, pails, perforated plastic bag and ventilated boxes, sterilized sand, germination box, thermohygrometer and other supporting materials.

Research implementation: Clone PB 260 seeds were harvested from Rubber Research Centre garden Sungei Putih, Galang, North Sumatera, Indonesia. Seeds were selected by Rubber Research Centre, seeds were mixed with moist sawdust and then put into jute. On arrival at faculty of agriculture seed technology laboratory, the seeds were washed several times with clean water. Then the shells were peeled and shelled seed and were directly selected. After that the shelled seeds were dipped for 10 min in a mix of PEG 6000 and fungicide solution according to the level of treatments and then dried for 6 h. After that, the seeds were stored in a plastic bag (with holes), arranged in a cardboard box, closed with some ventilation. Then stored at room temperature for 4, 8, 12 and 16 days. After storage, at each period the followings were observed: Fungi at various concentrations of PEG (%) and various doses of fungicide (%) and also the germination capacity (%). All data were then analyzed according to statistical experimental design.

RESULTS AND DISCUSSION

Effect of PEG 6000 during storage: The effect of PEG 6000 in various concentrations and analysis of variance results on fungal seeds during storage were shown in Table 1.

Table 1 showed that the PEG-6000 treatment at different storage periods had no significant effect on fungus seeds, the PEG of 30% was the best option to protect the rubber seeds from fungal attack. Storage at 30% PEG was able to suppress fungal attack. It can be seen that at 12 and 16 days of storage the fungal attack were only 18 and 42%, respectively. Seed cells have a specific osmotic value, as also do the PEG solution used for rubber seed storage media. The difficulty of water entering into the cells become increasingly large with increasing concentrations of PEG used so that the higher the potential drop of water in a medium (Steuter *et al.*, 1981). The PEG also indirectly helped suppress fungal growth in storage so it could help preserved the seeds in storage period. Toruan (1982) and Sutjiati and Saenong (2002) found that the fungal infections in seed storage were not influenced by PEG but by the metabolites that occurred in the metabolism of seeds and suspected borne diseases in the tissue or with the seed. Pathogen development was strongly influenced by the environment (temperature, humidity), nutrients, available water content and enzymes to break down tissue cells. Other than that the nature of parasitism between pathogenic organisms also could attacked each other to get nutrition from other pathogenic organisms. High humidity could help the formation of spores and increased infections.

Effect of fungicides during storage: Table 2, showed that the fungicide treatment and storage period had no significant effect at 4 and 8 days of storage. Whereas, at fungicides of 40 g the seeds were significantly protect from fungal attack during storage at 12 and 16 days (19 and 40%, respectively). According to Copeland and Mc-Donald (2001) and Walters *et al.* (2001), the process of respiration was very high so that the seeds had a fast metabolism and the heat generated makes the seeds moist, so that, they were easily contaminated with microbes and experiencing faster

Table 1: Average of fungus seed (%) during storage at various concentrations of PEG

	Storage period (days)			
Concentration of PEG (% w/v)	4	8	12	16
0	2[a]	10[a]	20[a]	42[a]
15	3[a]	14[a]	24[a]	38[a]
30	2[a]	10[a]	18[a]	42[a]
45	4[a]	12[a]	22[a]	46[a]
60	3[a]	12[a]	20[a]	58[a]

Different letters in a column showed significant effect in Duncan's multiple range test

Table 2: Average of fungus seed (%) during storage at various doses of fungicide

	Storage period (days)			
Fungicide (g/1 kg of seed)	4	8	12	16
0	5[a]	14[a]	29[a]	62[a]
10	3[a]	12[a]	21[b]	38[b]
20	1[a]	12[a]	17[b]	44[b]
30	3[a]	10[a]	19[b]	42[b]
40	3[a]	10[a]	19[b]	40[b]

Different letters in a column showed significant effect in Duncan's multiple range test deterioration. From of laboratory identification, dominant storage fungi were *Aspergillus* spp., *Penicillium* spp. and *Colletotrichum* spp. indicating that the pathogen infection occurred at the time of pollination, before harvest, at harvest, processing and seed storage for causing infection as the source of inoculum for the transmission of a disease. Fungal infected rubber seeds caused damage to important parts of the seed such as cotyledons, embryonic axis and radicle as a source of nutrition of pathogens, According to Pathak (1980), Parera and Cantliffe (1991) and Embaby and Abdel-Galil (2006), infection fungus could damaged seeds and membranes such as the pericarp, embryo and endosperm reduction ratio. Kabak *et al.* (2006), Christensen and Meronuck (1986) and Neucere *et al.* (1992) reported that *Aspergillus* spp. and *Penicillium* spp. were known as microbial contaminants and as an obstacle, especially in seed storage, could produced mycotoxins before and after harvested and were very carcinogenic and mutagenic. Galvan *et al.* (1997) said that the ability of pathogens anthracnose (*Colletotrichum* spp.) indirectly infected pathogens that occurred when an enzyme occurred and breaking the cell walls and infected the host. The presence of this fungus attacks were also found by Yuniarti *et al.* (2008) in ebony seed storage test. The failure of germination and fungus attack were suspected due to high water content of seeds in store that triggered an increase in fat acidity. Hydrolysis of the seed or the lipase activity of the fungus in a store space, made the seeds had chronological deterioration. Tune to reported Aziz and Mahrous (2004) that *Aspergillus* spp. produced aflatoxins can reduced lipid and carbohydrate content in wheat seeds, soy, beans and chili (Galvan *et al.*, 1997).

Interaction between PEG 6000 and fungicide during storage: In Table 3, the combined treatment that had the most moderate treatment was PEG concentration of 30% and fungicides of 40 g/1 kg of seed which able to protect seed from fungal attack during storage up to 18% after 16 days of storage. In the other treatment combinations PEG 6000 with concentrations of 30% and fungicides of 10 g/1 kg seeds was able to defend the rubber seeds from fungal attack in the storage up to 26%. It can be concluded that the concentration of PEG 6000 and fungicides with the right combination, has significantly defend rubber seeds from fungal attack in storage. Lian *et al.* (2004) and Emmerich and Hardegree (1990) mentioned that the effectiveness of PEG 6000 was able to inhibit imbibition and hydration of seeds in tune with the statement of Escobar *et al.* (2010) Lian *et al.* (2004) that the role of PEG osmotic potential properties and osmotic adjustment as a buffer to the water content of the seed and the passage of oxygen, could depress seed respiration and respiration heat, pressed the rate of deterioration and reduced moisture favored fungi. Pyraclostrobin, the active ingredient in the fungicide stops spores germination, preventing them from infecting plant tissue. By interfering with fungal cell energy production, pyraclostrobin causes the fungus to stop growing and die. Metiram, the active ingredient in fungicide is a nonsystemic, preventative fungicide that forms a protective barrier on the plants surface. When fungi try to

Table 3: Average of fungus seeds (%) on 16 days of storage in various conditions

PEG/fungicide (%)	0 g	10 g	20 g	30 g	40 g	Average
0	50^{bcde}	34^{bcde}	65^{bcd}	28^{cde}	35^{bcde}	42^{a}
15	44^{bcde}	37^{bcde}	28^{cde}	42^{bcde}	39^{bcde}	38^{a}
30	58^{bcde}	26^{cde}	65^{bcd}	44^{bcde}	18^{e}	42^{a}
45	66^{bc}	37^{bcde}	43^{bcde}	53^{bcde}	31^{cde}	46^{a}
60	96^{a}	55^{bcde}	19 de	45^{bcde}	78^{a}b	59^{a}
Average	63^{a}	38^{b}	44^{b}	42^{b}	40^{b}	45

Different letters in a row/column showed significant effect in Duncan's multiple range test penetrate this barrier, metiram disrupts multiple biological processes of fungal amino acid production, stopping fungal growth (The Chemical Company BASF, 2012). Fungicides could suppress the development of fungus by disturbing cell wall formation, cell membranes formation, protein synthesis and energy transformation reactions associated with the mitochondrial electron transport (Budiarti and Yulmiarti, 1997).

Table 4 and 5 showed that the germination of the rubber seed was close to 90% after storage for up to 16 days on each unit of PEG-6000 treatment and fungicide and seed germination test was continued for 21 days in a sandbox. At a concentration of 30% PEG 6000, 91% of seed germination were observed for 7 days and 97% at 14 days of observation. At 40 g/1 kg fungicide seed treatment, seed germination was 87% (7 days) and 88% at 14 days of observation. A relatively high percentage germination (±90%), when compared to the study of Sulaiman *et al.* (2010) with media store of moist sawdust which showed that rubber seed viability at 6 days of storage germination was 74% and at 12 days decreased was up to 59% and Sembawa Research Institute (2009) reported that a rubber seed storage for 14 days without the use of preservatives showed 0% germination. This is in consistent with the report of Charloq (2004) that showed that the 45% of PEG 6000 was able to maintain the viability of the rubber seeds up to 16 days of storage with 70% germination. Recalcitrant seeds storage was very complex and dilemma. Steuter *et al.* (1981) and Pammenter and Berjak (1999) reported that the cacao seeds (recalcitrant) could not be dried below 30% moisture content, where the seed would deteriorate and would not tolerant at low temperature. The indoor storage of clone DR2 with relative humidity of 35, 75 and 100% resulted in the decreased of fat content of the seeds, increased the fatty acid and sugar content and membrane leakage rate and the highest alcohol content was 35%. Sembawa Research Institute (2009) reported that a few days dryness would cause the seed not to grow. The decline of germination of stored seeds was related to the high water content which causes irregular structure of mitochondria

Table 4: Average of germinated seed (%) after storage at different concentration of PEG 6000

	Germination period (days)		
Concentration of PEG (% w/v)	7	14	21
0	90[a]	96[a]	96[a]
15	78[a]	81[a]	81[a]
30	91[a]	97[a]	97[a]
45	95[a]	97[a]	97[a]
60	86[a]	93[a]	93[a]

Different letters in a column, showed significant effect in Duncan's multiple range test

Table 5: Average of germinated seed (%) after storage at different doses of fungicide

	Germination period (days)		
Fungicide (g/1 kg of seed)	7	14	21
0	80[a]	94[a]	94[a]
10	87[a]	88[a]	88[a]
20	93[a]	96[a]	96[a]
30	92[a]	98[a]	98[a]
40	87[a]	88[a]	88[a]

Different letters in a column, showed significant effect in Duncan's multiple range test

Table 6: Average of germinated seed after storage (%) with different PEG 6000 and fungicide

PEG/fungicide (%)	0 g	10 g	20 g	30 g	40 g	Average
0	96	95	98	99	93	96
15	84	61	90	93	74	80
30	98	93	98	99	96	97
45	96	97	99	99	93	97
60	97	94	96	97	82	93
Average	94	88	96	97	88	93

membrane so that, membrane permeability increased. Increased permeability caused many metabolites (among others were amino acids and sugars) leaked out of fat cells. Thus the substrates for respiration were reduced so that energy produced for germination was reduced. This result is in consistent with the guidelines for the handling of recalcitrant seeds by Luna and Wilkinson (2009), that seed needed to have a constant gas exchange. The ability of PEG to retain water depends on its molecular weight and concentration (Sutjahjo *et al.*, 2007).

Ching *et al.* (1977), Barbedo and Cicero (2000) and Tatipata *et al.* (2004) showed that the seeds with high vigor contained ATP and total adenosine phosphate higher than those with low viability. ATP is necessary for the biosynthesis of new cells. The decrease in ATP is shown by lower germination. The result of this study was quite consistent with those mentioned by Harrington (1972) and Kartasapoetra (2003) who said that seed deterioration could not be avoided but could be reduced.

After treatment germination test: Table 6 showed the results of germination with several treatment combination.

The combined treatment of PEG 30% and fungicides of 40 g/1 kg of seed produced very high seed germination rate i.e., 96% (Table 6). Sulaiman *et al.* (2010) found that rubber seed germination in the early research was at average of 81% and after being stored for 18 days at 27-30°C decreased to 37 and 0% (Sembawa Research Institute, 2009).

It means that a substantial progress had been found. It can be recommended that the use of PEG can sustain the vigor and germination of rubber seeds at a high level. There are four fundamental difference of the above studies. Firstly seeds used in this study had been peeled, secondly, the seeds were treated with Polyethylene Glycol (PEG) as a preservative, thirdly, a period of storage was not the same and fourthly seeds were stored in a perforated plastic bag without using moist sawdust media at room temperature. Provision of PEG at a concentration of 30% had a significant interaction with fungicide of 40 g. They could maintain the germination above 90%. There are four advantages, resulting from the extraction of seed shells i.e., (1) Smaller storage space, (2) Reduce weight and delivery costs, (3) Seed can absorb PEG easily and (4) Easier seed quality evaluation.

CONCLUSION

PEG-6000 30% could maintain strorability of shelled rubber seeds on the storage period. Fungicides of 40 g/1 kg seeds had effectively inhibit the growth of fungi on the storage period. The combination of PEG-6000 30% and fungicide 40 g/1 kg of seeds was very effective in suppressing the fungal growth with 96% germination rate.

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