Proceedings of
The 3rd Annual International Conference Syiah Kuala University (AIC Unsyiah) 2013
In conjunction with
The 2nd International Conference on Multidisciplinary Research (ICMR) 2013
October 2-4, 2013, Banda Aceh, Indonesia

Maintaining viability of rubber shelled seed for storage of 16 days period by polyethylene glycol 6000 treatments

[1]Charloq, [2]Zulkifli Lubis, [3]Tumpal H. Siregar,[4] Sengly B. Damanik, [5]Elisa J. [6]Husni M., [7]Yazid A.

[1]Graduate Doctoral Agricultural Sciences Program, Agricultural Faculty, Sumatera Utara University, Medan 20155, Indonesia;
[2]Food Science and Technology Department, Agricultural Faculty, Sumatera Utara University, Medan 20155, Indonesia;
[3]Rubber Research Center, Sungei Putih, Galang 1415, Medan 20001, Indonesia;
[4]Agroecothechnology Department, Agricultural Faculty, Sumatra Utara University, Medan 20155, Indonesia;
[5]Food Science andTechnology Department, Agricultural Faculty, Sumatera Utara University, Medan 20155, Indonesia,
[6]Bachelor Agroecotechnology, Agricultural Faculty, Sumatra Utara University, Medan 20155, Indonesia;
[7]Master of Applied Statistics, Mathematics and Natural Sciences Faculty, Padjadjaran University, Bandung 40600, Indonesia.
Coorresponding Author: charloq@yahoo.com

Abstract. Rubber seeds have no dormancy, germinate rapidly and fungal growth is the limiting factor influencing storage period of seed viability. Solution of Polyethylene glycol 6000 (PEG 6000) have osmotic potential and osmotic adjustment that is used as a coating material is expected to be a preservative during storage. The purpose of this study was to test the effect of polyethylene glycol 6000 in inducing secondary dormancy by reducing the seeds germination and fungus growth to maintain the seed viability in storage period and to test seed viability and vigor after storage period. First stage of the study was using non-factorial completely randomized design with four levels of polyethylene glycol with four replications, namely: PEG 6000 (w / v): 0%, 15%, 30%, 45%; The second step, was germination phase in germination tub after the storage period. The first study showed that concentration of 15% PEG 6000 to 45% were able to induced secondary dormancy by delaying seed germination from 0.67% to 0.33%, that significantly different from the 0% PEG 6000 (17.67%) that was able to reduced fungal seed from 16.33 to 26.33% and was not significantly different in each treatment. The second study showed that PEG 6000 can maintain viability and vigor of seeds with seed germination above 97%, seedlings height 22.40 cm and number of leaves was 7.16. PEG 30% is the best concentration to increase the shelf life period of rubber seed and maintained morphological characteristics after storage period. The higher the concentration of PEG 6000 the higher were the seed viability during shelf. The morphology and growth of the seedlings, had the same adaptability to the tub germination in planting medium.
Keywords: rubber seeds; recalcitrant; storage; polyethylene glycol 6000; seed viability.

Introduction

The storage period is a function of time, then the difference between orthodox and recalcitrant seeds was based on its ability to the storage period (Botha and Small, 1985). Depletion of food stocks through respiration in recalcitrant seeds may resulted in faster store seed germination leading to premature aging (deterioration) and the loss of seeds energy to grow in the field. According to Emmerich and Handegree (1990) solution of polyethylene glycol (PEG) is commonly used to control water potential in the study of seed germination. PEG is an inert chemical and non-toxic so it does not damage the seed metabolism. Therefore the treatment of polyethylene glycol (PEG 6000) was aim to prove the effect of PEG 6000 in inducing secondary dormancy by delaying seed germination and pressing the growth of fungus and to test the viability and vigor of seed germination phase after the storage.

Materials and Methods

Research was conducted at Seed Technology Laboratory and Food Science and Technology Laboratory from January to March 2012 at Agricultural Faculty, Sumatera Utara University, Medan, Indonesia. Materials used were clone PB 260 rubber seed (moisture content of 45,73%), polyethylene glycol with a molecular weight of 6000 (PEG 6000), fungicides (*a.i*) pyraclostrobin + metiram (40 g/1kg seed), insecticide Sevin 80S, distilled water, 70% alcohol, seed storage cardboard boxes. Research equipment used were: analytical balance, perforated plastic bag and ventilated boxes, sterilized sand, germination box, thermohygrometer and other supporting materials. The first stage of the research was methods using non-factorial completely randomized design with four replications, i.e: PEG 6000 (w/v): 0% (P0), 15% (P1), 30% (P2) and 45% (P3). Seeds that have been getting treatment stored in 16-day period. Parameters for the storage period were germinated seeds (%) and fungal seeds (%).The second stage of the research was using completely randomized design non-factorial, with four variations of seed (seed that comes from the first stage) with four replications. Parameters observed were seed germination (%), seedling height (cm) and number of leaves of the seedlings (strands).

Research Implementation

The seeds were washed several times with clean water, then shelled to selected more carefully and the selected seeds was healthy seed that has solid white and unblemished endosperm.

PEG 6000 preparation and seed treatment

PEG 6000 was weighed according to treatments. Each samples was dissolved in 1 liter of aquadest and added with proper fungicide dose has been determined and stirred until evenly distributed. Seeds were dipped for 10 minutes into a solution of PEG 6000 and then filtered and wind dried for 6 hours on a paper panels in the laboratory. The seeds were then put in a perforated plastic bag, and arranged into a perforated cardboard boxes and closed, after that stored in a room with room temperature for 16 days. After a storage the seeds germinate (%) and seeds contact with fungi (%), was determined. The next second stage, The germinated seeds were transferred into a bed with sterile sand, in Seed Technology Laboratory room. After 21 days of planting were observed: The power germinated seeds (%), plant height (cm) and leaves number (strands). All data were analyzed statistically.

Results and Discussion

Effect of PEG 6000 on the percentage of germination (16 days storage)

Table 1. Average seed germination (%) in storage at various concentrations of PEG 6000

PEG 6000	Replications				Average
	I	II	III	IV	
0% PEG	14.67	17.33	21.33	17.33	17.67[a]
15% PEG	0.00	0.00	2.67	0.00	0.67[b]
30% PEG	0.00	0.00	0.00	1.33	0.33[b]
45% PEG	0.00	2.67	0.00	0.00	0.67[b]
Average	3.67	5.00	6.00	4.67	4.83

Description: The numbers that were not followed by the same letter in the row between treatments, showed significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level.

Table 1 showed the measures save seeds germinated in the period, all PEG 6000 treatments indicated lower germination than seeds without PEG. PEG had highly significantly maintained seeds that did not germinate during the storage. Concentration of 30% PEG can pressed the seeds germinate in storage until 0.33% and differ significantly with the 0% PEG (17.67%). The increase in seed viability during storage as controlled by the potential osmoticum PEG 6000 by reducing water imbibition into the seed. Seed imbibition is a requirement for seed germination, while the rubber membrane permeable seed is easily through by water and gas (Gosling, 2007). Supported by Steuter (1981) telling that the difficulty of imbibition of water into the larger cells with the increase of concentration of PEG resulted in the higher the potential drop of water in the medium. Consistent with reports of Escobar (2010) that the role of PEG was to adjustment the osmotic potential properties Song, 2011 used PEG in seed storage medium to limit the availability of water and oxygen.
Opening of rubber seed shell (Charloq, 2004) at the time of seed conservation did not only helps in improving the selection of rubber seed in conventional systems but also help the effectives of seed to absorb the osmotic solution and simplify the attachment of the solution on the surface of the seed itself, and could function as a replacement of seed shell (seed coating). Therefore the PEG 6000 as a seed coating was able to induce secondary dormancy through seed preservation in maintaining seed viability and vigor.

Effect of PEG 6000 on the percentage of air fungal seed during storage for 16 days

Table 2. Average of fungus seed (%) during storage for 16 days at various concentrations of PEG 6000

PEG 6000	Replications				Average
	I	II	III	IV	
0% PEG	14.67	25.33	16.00	9.33	16.33[a]
15% PEG	20.00	25.33	20.00	40.00	26.33[a]
30% PEG	24.00	21.33	26.67	6.67	19.67[a]
45% PEG	32.00	33.33	18.67	16.00	25.00[a]
Average	22.67	26.33	20.33	18.00	21.83

Description: The numbers that were not followed by the same letter in the row between treatments, showed significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level and the upper level of 1%.

Table 2 shows that the air fungal had no significant effect so that the PEG can press the growth of fungi, especially with PEG 30% (19.67%) which were not significantly different from 0% PEG (16.33%). PEG integrated with fungicide (40g/1kg seed) can reduce fungi attacks below 30%. PEG 6000 are integrated with fungicide (ai phyraclostrobin-metiram) can help suppress the proliferation of fungi in storage (The Chemical Company BASF, 2012). Results of this study were better compared to Sulaiman, et al. 2010, which used the storage temperature of 20°C - 22°C and temperature of 23°C - 26°C with media store of sawdust moist. The rubber seed viability on the 6 days of storage was 74.44% germination. 12 days, 59.33%, 18 days decreased to 40.22%. Vesselina, et al. 2003 reports so that no one had found a data strategy to maintain the seeds from fungal attack on recalcitrant seeds storage period.

Effect of PEG 6000 on seed germination after storage

Table 3. Average of test seed germination (%) after period of storage at different concentrations of PEG 6000

Seed Variations	Replications				Average
	I	II	III	IV	
0% PEG	97.33	100.00	98.67	98.67	98.67[a]
15% PEG	94.67	97.33	100.00	97.33	97.33[a]
30% PEG	100.00	100.00	98.67	100.00	99.67[a]
45% PEG	97.33	100.00	98.67	98.67	98.67[a]
Average	97.33	99.33	99.00	98.67	98.58

Description: The numbers that were not followed by the same letter in the row between treatments, showed significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level and the upper level of 1%.

Table 3 showed that the average of seed germination was highest at 30% PEG treatment ie 99.67%, which was not differ significantly with 0% PEG (98.67%), 15% PEG (97.33%), and 45% PEG (98.67%). These results showed that PEG treatment could maintain seed viability during storage with a high germination rate. Rubber seeds have a high water content 45.73%, temperature and high water content is a deminishing germination factor. The reduced of seeds germination associated with a high water content that causes irregular mitochondrial membrane structure that increased membrane permeability. The increase in permeability resulted in many metabolites such as sugars, amino acids and fat leaked out of the cell and ultimately seed will deteriorate (Zhang and Xiaofeng, 2004). In this case PEG 6000 can preserve the viability of seed germination.

Rubber seedlings height (cm)

Table 4. Average height of rubber seedlings (cm) after storage (16 days) at 21 days after planting

Seed Variations	Replications				Average
	I	II	III	IV	
0% PEG	24.53	23.62	22.96	21.82	23.23[a]
15% PEG	22.08	23.72	22.02	24.29	23.03[a]
30% PEG	22.16	17.84	22.03	22.53	21.14[a]
45% PEG	19.40	22.40	24.69	22.33	22.20[a]
Average	22.04	21.90	22.92	22.74	22.40

Description: The numbers that were not followed by the same letter in the row between treatments, showed significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level.

Table 4 shows that is no siqnificant difference among all PEG treatment. PEG 6000 gave good growth of the rubber plant seeds. The second stage show that the stored seed that moved into the tub planting germination, have the same adaptability to the environment growing media. These results are supported by Bambang & Bambang (2007) which forward that the seeds that have high viability will affect subsequent seedling growth and development.

Leaves number at 21 days after planting (pieces)

Table 5. The average number of leaves (pieces) of the stored seeds after 21 days planting

Seed variations	Replications				Average
	I	II	III	IV	
0% PEG	7.44	7.49	7.44	8.13	7.63 [a]
15% PEG	6.53	6.33	6.47	7.41	6.69 [b]
30% PEG	7.38	7.33	6.72	7.06	7.12 [ab]
45% PEG	6.82	7.24	7.36	7.40	7.20 [ab]
Average	7.04	7.10	7.00	7.50	7.16

Description: The numbers are not followed by the same letter in the row between treatments, showed significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level.

Table 5 showed that the development of leaves number of PEG-treated seed resulted had no significant difference, but significantly different from 0% PEG seed (7.63 pieces). The effect of PEG still bound to leaves development, in consistent with Song (2011) that used PEG in seed storage to restrict the water and oxygen availability, but the development of leaves not permanently inhibited. According to Botha (1985), the PEG ability to maintain food reserves proved that seed can still save energy supply that will be used by the embryo to grow and develop.

Conclusion

PEG 6000 was able to induce secondary dormancy of seeds to delay seeds germination up to 0.33% and pressed the fungal attack up 19.67% at concentration of PEG 30%. PEG 6000 was able to maintain seed viability after storage with seed germination rate above 97.33%, and sustain the growth morphology. PEG 6000 30% was the best concentration to increase storability of rubber seed and maintain the morphological properties after storage during the period.

Acknowledgements

The authors thank the Rubber Research Institute Centre Sungei Putih that supported this research.

References

Bambang B. S and Bambang S. P, 2007. Study on the technic of physic nut (*Jatropa curcas* L) seedling: The effect of seed storage and transplanting date to seedling growth. Agroteksos Vol.17 No.2 Agustus 2007. http://fp.unram.ac.id/data/2012/04/02-Studi-Teknik-Pembibitan_Bambang-B.pdf

Botha, F. C. and Smal., J.G. C., 1985. Effect of Water Stress on the Carbohydrate Metabolism of Citrullus lanatus Seeds during Germination. *Plant Physiol*. (1985) 77, 79-82.

Charloq, 2004. (*In Indonesian language*) Upaya Peningkatan Ketahanan Simpan Dua Variasi Benih Karet (*Hevea brasiliensis*, Muell - Arg) Dikupas Melalui Pemberian Polyethylene Glycol. *Tesis*. Program Pasca Sarjana Universitas SumaterabUtara. *USU repository* 2008. http://repository.usu.ac.id/handle/123456789/3891.

Emmerich W. E. and S. P. Hardegree. 1990. Polyethylene glycol solution contact effect on seed germination. *J. Agron*. ,82 1103-1107.

Escobar, H., R. Bustos, F. Fernández, H. Cárcamo, H. Silva, N. Frank and L. Cardemil, 2010. Mitigating effect of salicylic acid and nitrate on water relations and osmotic adjustment in maize, cv. Lluteño exposed to salinity. *Cien. E Invest. Agrar.,* 37:71-81.

Gosling, P., 2007. Raising Trees and Shrubs from Seed. Forestry Commission Practice Guide. First published in 2007 by the Forestry Commission. 231 Corstorphine Road, Edinburgh EH12 7AT. ISBN 978-0-85538-736-5. Forestry Commission, Edinburgh. i–iv + 1–28 pp. Printed in the United Kingdom.

Song A. N, T. D. Colmer, L. J. Wade, and G.Cawthray, 2011. Osmotic Adjustment and Solutes Accumulation in Leaves of Wheat (*Triticum aestivum* L.) during Water Deficit. *Jurnal Matematika dan Sains*, April 2011, Vol.16 Nomor 1.

Sulaiman, F ., Aaron, M. Umar and A. Kurniawan, 2010. (*In bahasa*) Perkecambahan benih tanaman karet *(Hevea brasiliensis* Muell. Arg) yang disimpan pada suhu dan periode yang berbeda. ISBN 978-602-98295-0-1. Prosiding the National Seminar on Outcomes Research and Assessment, December 13-14, 2010, Research and Development of Provinece of South Sumatera, Palembang.

Steuter, A.A., A. Mozafar and J. Goodin, 1981. Water Potential of Aqueous Polyethylene glycol. *Plant Physiol* 67: 64-67.

The Chemical Company BASF, 2012. Cabrio plus fungidice potato technology sheet. AgSolutions® by BASF at 1-877-371-BASF (2273).

Vesselina, S.A-M, Calistru, C. and Berjak, P., 2003. A Study of Some Biochemical and Histophatological Responses of Wet-stored Recalcitrant Seeds of *Avicennia marina* Infected by Fusarium moniliforme. School of life and environmental sciences University of Natal, Durban, 4041 South Africa. *Annals of Botany* 92: 401 – 408, 2003.

Zhang Ming and Wang Xiaofeng, 2004. Effect of osmoconditioning on mitochondrial respiration and phosphorylation in Soybean seeds. Forestry Studies in China , 6(4): 8-12.