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Research Article

Physiology Changes of Shelled Rubber (*Hevea brasilliensis* Muell. Arg.) Seed After 16 Days Storage with PEG 6000 30% Coating to Induce Secondary Dormancy

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Abstract

Maintaining a physiological seed quality in storage period is necessary. Deterioration of rubber seeds during storage period is a decrease of physiological quality that causes physiological changes in seed that reduce seed viability. Rubber seed has a relatively high water content, respiration and metabolism and remained active during storage period. The PEG 6000 has an osmotic potential, that can be used in seed storage period to limit changes in water content and oxygen in the storage medium that can induce secondary dormancy. The purpose of this research was to examine the physiological change of rubber seed after stored with PEG 6000 for 16 days to induce secondary dormancy in maintaining seed viability. This study examined differences in treatment with and without PEG 6000 30% with 4 replications using t-test at $\alpha = 5\%$. Parameters observed were fungal seed found in storage (%), seed germination (%), moisture content (%), water activity (aw), O_2 consumption (mL kg^{-1} jam), CO_2 production (mL kg^{-1} jam), total sugar content (%), protein content (%), ash content (%), fat content (%), electrical conductivity (μmhos cm^{-2} g^{-1}), peroxide value (%), seed hardness (kgf) and germination of seeds after storage period (%). The results showed that the physiology of shelled rubber during storage for 16 days with the PEG 6000 30% coating would be changed not significantly with a viability of 99%.

Key words: Physiology of shelled rubber seeds, PEG 6000, recalcitrant seeds, secondary dormancy, storage, viability

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

During storage, seeds will experience aging and deterioration. Seeds are retreating, the respiration rate increases which causes a reduction in food reserves and can starve the meristem tissue. Seed deterioration can be observed from the decline of physiological seed quality which can lead to overall changes. Pammenter and Berjak (2014) stated that desiccation-sensitive (recalcitrant) seeds cannot tolerate water loss and so cannot be stored using conventional seed bank conditions. Particularly with respect to storage, recalcitrant seeds do not undergo intracellular dedifferentiation nor any significant metabolic shut down. Setbacks indication such as seed moisture reduction, respiration rate, decreased food reserves, increasing of seed conductivity value and germination decline were found after the storage period. The physiology of seed can be influenced by seed quality that have been through the stages of storage process. Rubber seed belong to recalcitrant seeds, based on observations of its behavior pattern, Pammenter and Berjak (2000), Tweddle *et al*. (2003) and Berjak and Pammenter (2013) stated that the recalcitrant seeds had no dormancy, had a short life in storage, having undergone drying when physiologically ripe, apart from the water content that relatively high, ranging between 30-70%. Berjak and Pammenter (2008) and Pukacka and Ratajczak (2006) in Berjak and Pammenter (2013) reported that the recalcitrant seed storage, was a dilemma, very complex and could not be dried to below 30% moisture content and would be damaged and not tolerant to low temperatures. In these conditions, the metabolism still active and ongoing process towards germination even in the resting state (quiscent). When the seeds were dried (desiccating) water content decreased and sub-cellular changes began to occur, resulting in deterioration of seed viability that declining rapidly. Berjak and Pammenter (2004) stated that no single technique was adequate for storing recalcitrant seeds for a long period. Charloq *et al*. (2013) have reported that recalcitrant shelled rubber seed germination during storage can be inhibited by coating the seed with the PEG 6000.

According to Faria *et al*. (2006) storage of hydrated embryos in a solution of polyethylene glycol (PEG) at -1.7 MPa water potential was capable of maintaining high germinability until 30 day of storage. So does the solution of polyethylene glycol (PEG) which is used for recalcitrant seed storage media. Polyethylene glycol has a cell osmotic potential, which can be used to limit changes in water content and oxygen on germination medium or storage so that the PEG molecules, that is outside the cell seed membrane will form a film which protects the seeds and also functions as a buffer for seed moisture content and oxygen. This was supported by Ayranci and Sahin (2008) and Chao *et al*. (2012), who stated that PEG ($HO\text{-}CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)n\text{-}CH_2\text{-}OH$) is a long-chain polymer compound, inert, non ionic and non-toxic and not affect the metabolism of seeds and seed physiological function after a storage period. Physiological process deterioration after a storage period can cause an overall change in the content of seeds and reduce the viability of seeds and have an impact on the next phase of life. Therefore, a study on shelled rubber physiology changes using PEG 6000 30% after storage to induce secondary dormancy was needed to maintain shelled rubber seed viability.

MATERIALS AND METHODS

This research was conducted in Laboratory of Seed Technology and the Laboratory of Food Technology in January-March 2012 at the Faculty of Agriculture, University of North Sumatra, Medan. Materials used in this research were PB 260 rubber seed (certificate of Rubber Research Institute, Sungei Putih), polyethylene glycol 6000 (code 8.07491.1000 1 kg, Merck Schuchardt OHG 85 662 Hohenbrunn, Germany) (Cabrio Top 60 WG, a.i. metiram complex+pyraclostrobin, BASF) 40 g/1 kg seed, Sevin 80S (a.i. carbaryl 85%, Bayer), distilled water, alcohol 70 and 95% (Merck), deionized water/distilled water, catalysts, $BaCl_2$ $2H_2O$ (Merck), methyl blue (Merck) 0.02%), HCl (Merck) 0.002 N, glacial acetic acid (Merck), chloroform (Merck), potassium iodide (Merck), sodium thiosulfate (Merck) 0.1 N, concentrated H_2SO_4 (Merck), NaOH (Merck) 50%, 0.02 N NaOH (Merck), Pb-acetate solution (Merck), Na_2CO_3 (Merck) 8%, KOH (Merck) 0.1 N, hexane (Merck) and phenolphthalein indicator (Merck), charcoal. Equipment used were a ventilated cardboard box and perforated plastic bag for seed storage. Seed shell peeler. Laboratory tools, analytical balance (Electronic Precision Balance, ACIS Ad-600H), oven (Mammert), hand sprayer (Swan), measuring cup (Pyrex), germination box, sterile sand, thermohygrometer (Digital Thermo-Hygrometer Brand TFA) and erlenmeyer glass (Pyrex) 100 and 250 mL.

Research methods: This study examined differences in treatment with and without PEG 6000 30% with 4 replications using t-test at $\alpha = 5\%$ level (Steel and Torrie, 1995).

Research implementation: Freshly harvested PB 260 seeds that have been selected visually including resilience, shiny shell color and water floating were mixed with moist sawdust (1:1) and put in jute and packed with wooden crates. At arrival in Seed Technology Laboratory, Agriculture Faculty, USU, the seeds were washed several times with water, air dried and

then shelled for more accurate selection of seed endosperm. Polyethylene glycol 6000 (PEG 6000) were weighed in accordance with the level of treatment, dissolved in 1 L of distilled water and mixed with a fungicide (a.i pyraclostrobin+metiram) at 40 g L^{-1} kg of seeds. Seeds were dipped for 10 min in PEG 6000 solution according level of treatment and then wind dried for 6 h on paper panels in the laboratory. The seeds were then stored in a perforated plastic packaging and perforated cardboard boxes and then closed. After that stored in the laboratory at room temperature with max temperature of 31.5°C. The average daily RH was 70.81 (RH min 53-max 83).

Laboratory analysis

- Percentage of fungus seed in storage (Don, 2003)
- Percentage of germinated seeds in storage (Don, 2003)
- Moisture content (AOAC., 1995)
- Water activity (aw) (AOAC., 1995)
- Seed respiration rate (Deily and Rizvi, 1982)
- Total sugar content (%) (AOAC., 1995)
- Protein content (%) (AOAC., 1995)
- Ash content (%) (AOAC., 1995)
- Fat content (%) (AOAC., 1995)
- Free fatty acid content (%) (AOAC., 1995)
- Peroxide value (%) (AOAC., 1995)
- Electric conductivity (μmhos cm^{-1} g^{-1}) (AOSA., 2002)
- Fruit hardness tester (kgf)
- Germination seed test after storage (%) (Don, 2003)

RESULTS AND DISCUSSION

Table 1 showed the results of the experiment. The PEG 6000 30%, can maintain rubber seed viability without shell with 99.67% germination after 16 days storage.

This result is an improvement when compared with the report of Tabin and Shrivastava (2014) that the recalcitrant seeds of *Aquilaria malaccensis* Lamk. possess short viability period. Nursery trials were conducted to examine the effect of storage periods (four treatments) and light intensity (three treatments) on Aquilaria seed germination and growth. The maximum germination was obtained with fresh sown seeds (92.0%) followed by 5 days (39.6%) and 10 days (4.0%) stored seeds. No germination took place in 15 days stored seeds at all. According to Kozeko and Troyan (2000), Tweddle *et al.* (2003) and Pammenter and Berjak (2014), recalcitrant seeds have a high water content after harvest, does not have dormancy period and low storability because of respiration. When desiccated, the moisture content decreased and sub-cellular changes started to occur, resulting in deterioration of seed viability. PEG 6000 30% can maintain or reduce the rate of seeds deterioration so that physiological changes that happen in the seed becomes insignificant. It can be seen from the Table 1 that there was 19.67% fungal seeds and only 0.33% of the seeds were germinated. The water content before storage period was 50.28%, dropped significantly to 35.71%, might be due to very high respiration as a result of heat generated which made the seed to moist and contaminated by fungal. According to Berjak and Pammenter (2008) recalcitrant seeds should be stored in as hydrated a condition as when they are shed, such hydrated storage has attendant problems of fungal proliferation which, unless minimized, will inevitably and significantly affect seed quality. In these conditions, the metabolism still occur and was process towards germination. Seeds can not be drained because it will be damaged, so it can not be stored in a dry environment condition. Water content was too low suspected that damage cellular components sub and change the structure of enzymes, structural proteins and a decrease in cell membrane integrity. The cell wall became impermeable, making it is more difficult to imbibite water, diffused pass through the membrane and inhibited the metabolic activity in the seed. Seed damage happened as a result of the consequences of an unbalanced metabolism during dehydration and when stored in a hydrated condition (Golovina *et al.*, 2010; Berjak and Pammenter, 2013).

No significant changes, happened in aw (0.60-0.57). The PEG 6000 30% could suppress the increase in the amount of free water in the seed during storage. This result was also found by Pardo *et al.* (2006) and Astoreca *et al.* (2010), who stated that the fungus that often affects seed storage period were *Aspergillus* spp., *Penicillium* spp. and *Colletotrichum* spp. The minimum water activity for multiplication of *Penicillium* spp., was at aw 0.83-0.85, *Colletotrichum* spp. and *Aspergillus* spp., were at 0.77-0.83. The PEG 6000 (30%) was suitable for seed preservation process because it has the ability to bind water in the seed. It might be due to water infiltrate through the thin membrane, so that water will flow freely in the seeds of less concentrated solution to a more concentrated solution passes through the membrane (Copeland and McDonald, 2001; Gekas *et al.*, 1998; Hussain *et al.*, 2015).

Oxygen consumption increased from 68-138 mL kg^{-1} h^{-1} and CO_2 increased from 91.13-256.92 mL kg^{-1} h^{-1}. Increased of O_2 consumption and CO_2 production showed a significant increase in carbohydrate oxidation through respiration which was a characteristic of rapidly least compositional changes that happened in seed tissue. The PEG 6000 30% through osmotic potential, encourage seed impermeability, to withstand water imbibition, diffusion of oxygen and increase carbon dioxide that can reduce the oxygen consumption and carbon dioxide production that suppress respiration. Lowering

Table 1: Physiological parameters of treated shelled rubber in PEG 0 and 30% seed before and after 16 days storage period

Parameters	PEG 0% 0 day	PEG 30% 16 days
Fungus seed (%)		
C.I for mean	-	19.67±14.22
Significance	-	
Germinated seed (%)		
C.I for mean	-	0.33±1.06
Significance	-	
Moisture content (%)		
C.I for mean	50.28±6.07	35.72±2.55
Significance	*	
Water activity (aw)		
C.I for mean	0.60±0.20	0.58±0.06
Significance	ns	
Respiration rate of O_2 consumption (mL kg^{-1} jam)		
C.I for mean	68.27±0.89	138.48±2.98
Significance	*	
Respiration rate of CO_2 production (mL kg^{-1} jam)		
C.I for mean	91.13±2.22	256.92±9.05
Significance	*	
Total sugar content (%)		
C.I for mean	4.73±0.82	5.01±0.46
Significance	ns	
Protein content (%)		
C.I for mean	12.69±0.81	12.69±0.81
Significance	ns	
Ash content (%)		
C.I for mean	3.76±0.73	3.76±0.93
Significance	ns	
Fat content (%)		
C.I for mean	8.25±0.58	9.44±0.57
Significance	*	
Free fatty acid content (%)		
C.I for mean	0.34±0.21	5.78±2.91
Significance	*	
Electric conductivity (μmhos cm^{-2} g^{-1})		
C.I for mean	1.72±0.06	2.92±0.37
Significance	*	
Peroxide value (%)		
C.I for mean	1.71±0.35	2.94±0.86
Significance	*	
Seed hardness (kgf)		
C.I for mean	3.65±0.43	4.12±0.10
Significance	*	
Germination seed test after storage (%)		
C.I for mean	98.33	99.67±1.06
Significance	ns	

*: Significant effect, ns: Not significant effect in t-test

the rate of respiration until minimum will be able to extend the economic life. This was supported by Pammenter and Berjak (2000), Booth and Sowa (2001), Caccere *et al.* (2013) and Barbedo *et al.* (2013), who found that the increase in permeability caused many metabolites including sugars, amino acids and fats to leak out of the cell. Thereby substrates for respiration in storage period was reduced so that the energy generated to germinate was reduced. The PEG 6000 30% could create an impermeable cell with isotonic conditions approached the same osmotic pressure inside and outside the cell.

The increase in total sugar content was not significant (4.73-5.08%). Maintaining sugar content of recalcitrant seeds in storage is difficult, because the rate of respiration at high water levels was very high. Decrease seed quality is correlated with the length of rubber seed stored due to respiration process which resulted in nearly all food reserves (proteins, fats and carbohydrates) could be reduced. A decrease in the sugar content of the seed will decrease seed viability. However, the existence of the sugar component was also found by Corbineau *et al.* (2000) in Bailly *et al.* (2004), who stated that the effects of dehydration on desiccation

sensitivity in touch containing oligosaccharides and cell membrane material, in the metabolism of carbohydrates, sugar is a stabilizing agent, sucrose involved in preventing liquid crystalline into a gel phase transition and oligosaccharides act as preventive effect of crystallization.

The protein content was not significantly different (12.69-12.84%). These results demonstrated that the viability of the seeds could be maintained by the PEG 6000. The PEG had a function as an osmotic agent, protects the membrane and prevent damage to structures and protein denaturation on storage conditions, that useful in inducing secondary dormancy. Increasing amount of proteins was considered as indication of stress tolerance to storage, as the ability of PEG to hold water. According to Tatipata (2009), high moisture content in seed could cause increased on protein damage. The results of this study supported by Braccini *et al*. (2000), that soybean seed storage protein reduced periods but PEG 6000 treatment showed lower protein reduction. Salah *et al*. (2015) reported that the performance of the molecules of PEG 6000 was to improved the cell structures, suspected that the molecules of PEG 6000 was to forms a thin layer that protects and serves as a buffer to moisture and oxygen ingress, as also found by Sung and Chiu (2001), who stated that a protein was associated with the return of detoxification enzyme activity by controlling the rate of lipid peroxidation.

The decrease of ash content was not significant (3.76% preserved to 3.71%). Therefore, 30% PEG 6000 can maintain the stability of the ash content of the seed, which means that the higher the ash content of the food, the worse the quality of food (Schuck *et al*., 2012).

The fat content was significantly increased from 8.25-9.44%. During the storage period of 16 days, the PEG 6000 30% showed a very significant role in maintaining the high fat content of the seed, as also found by Rohandi and Widyani (2011) which stated that the fat content in recalcitrant seeds tend to increase consistently with decreasing of water content.

The free fatty acid levels increased significantly from 0.34-6.06%. The existence of free fatty acids in the rubber seeds is one of the indicators of quality defects. Along with the length of the seed in storage free fatty acid levels was increase. This also showed by Osawa *et al*. (2007), Canakci and Munzuroglu (2007) in Enteshari and Sharifian (2012), Berchmans and Hirata (2008) and Akowuah *et al*. (2012), who reported that the increase in free fatty acids was correlated with high levels of seed moisture during the storage period. It seemed that free fatty acids increased was due to hydrolysis of triglycerides by high humidity and oxygen during seed storage period.

The electrical conductivity increased significantly from 1.72-2.82 μmhos cm^{-1} g^{-1}. Treatment with PEG 6000 30% in the mechanism of osmotic potential properties is expected to reduce imbibition to prevent dehydration that weakened/soften the seed cell membrane structure. Vieira *et al*. (2004), Fessel *et al*. (2006), Setyowati (2009), Ramos *et al*. (2012) and Navaey *et al*. (2014) stated that the electrical conductivity of seed has a high sensitivity in detecting deterioration of seed. Matthews and Powell (2006), Don (2003) and Abdellaoui *et al*. (2013) reported that the higher the electrical conductivity of the test results, the lower the seed vigor. This is because the increase in electrical conductivity caused by electrolyte leakage due to increased seed membrane permeability.

A significant increase was found in peroxide value from 1.71-2.84%. The high peroxide value may be caused by contact with air and storage duration, so that the oxidation reaction can take place. Oxidation process can take place when there is contact between the amount of oxygen with rubber seeds that have a high fat content. Rubber seed fat composition was high at 45.63% (Shokib *et al*., 2010), the high fat content in the rubber seeds accelerated peroxide of unsaturated fatty acid compounds, Ikwuagwu *et al*. (2000) found that the unsaturated fatty acids can bind oxygen to the double bond to form peroxides. Peroxide is one important measure to determine the degree of damage to the seed. In this study, the rubber seed was stored at room temperature of for 16 days and the seeds still have a relatively small number of peroxide, which is supported by standard of APCC (2005), peroxide value (2.94%) is still relatively safe for food, indicate that the quality of the oil or fat was very bad, usually identified from an unpleasant smell. Varier *et al*. (2010) stated that deterioration of seed was caused by damage of cell membranes and other cellular components. Peroxide is usually used as a measure of rancidity, suspected oxidative rancidity rate, increases with increasing storage time. This is supported by De Alencar *et al*. (2010), who stated that the peroxide and photometric color index increased significantly, independently of storage conditions; however, the increase was more accentuated in oil extracted of stored grains at high moisture content and temperature.

From this study it was found that PEG 6000 30% can maintain a peroxide value of the seed, in relatively small amount. Inhibition of the oxidation process in the seed was intended to make seed more resilient and not easily deteriorate during storage. Bailly *et al*. (2000) reported that PEG could control the peroxide-free and the cessation of lipid peroxidation processes with the increased of protein associated with the active detoxification enzymes that control the rate of lipid peroxidation through H_2O_2 cleaning.

Seed hardness increased significantly from 3.65-4.14 kgf during storage. The addition of PEG 6000 30% coating increased the inhibitory ability against the impairment of seed hardness. The PEG 6000 30% coating which approach isotonic concentration of seeds was also expected to hold water so that the transmission rate can be retained, that was supported by Wu (2002), Li *et al.* (2013) and Konarska (2013), who stated that the rate of seed damage was influenced by gas diffusion into and out of the seed that occur through lenticels that spread across the surface of the seed and naturally inhibited by contained wax coating on the surface of the seed. It seemed that the decreasing of hardness values in the seed during storage was due to the reduction in germ cell turgor pressure, which also supported by Gonzalez *et al.* (2010) which stated that the seeds of onions violence influenced by turgor of cells are still alive and according to Banjongsinsiri (2003) was caused by the loss of turgor pressure, reshuffle and degradation of starch into glucose so that the cell walls increasingly softened.

The germination ability of the seeds was 99.67% which was not differ significantly from the fresh seeds (98.33%). Germination of seeds is one of the parameters that are directly describe seed viability. Viability of seeds tend to decrease when the seeds are in storage. This is supported by Fotouo-M *et al.* (2015), who that seed ageing during storage is one of the main causes of reduction in seed quality and this results in loss of vigour and failure to thrive. The decrease in seed viability during storage was associated with a loss in membrane integrity which was evidenced by an increase in electrolyte leakage. As reported by Tatipata (2009) that the decline in germination of seeds after storage period was associated with high level of water that causes the irregular structure of the mitochondrial membrane, thus increasing membrane permeability. Increased permeability might cause many metabolites including sugars, amino acids and fatty leak out of the cell. Thus substrates for respiration is reduced so that the energy generated to germinate was reduced. The results of this study have also been evidenced by Charloq *et al.* (2013), who found that the combination of PEG 6000 30% and fungicide 40 g/1 kg seed rubber without shells stored for of 16 days was 96.00% germinated. The PEG 6000 30% was successfully induce dormancy of seeds during storage for 16 days, by reducing the rate of deterioration. These results were supported by Bailly *et al.* (2000) and Sung and Chiu (2001), who stated that PEG 6000 through the mechanism of osmoregulation water replacement hypothesis and various protective mechanisms can address the causes of oxidative damage and maintain the water content, water activity, respiration rate, total sugar content, membrane leakage and physiological function of the seeds so that germination of seeds can be maintained. The PEG 6000 30% was successfully preserve the viability of the seeds by high germination rate, compared to Tabin and Shrivastava (2014), who reported that the maximum germination (78.0%) obtained under Double Layer Polynet Shade (DLPS) as compare to Single Layer Polynet Shade (SLPS) and control (75.0 and 63.8%, respectively).

CONCLUSION

The observation of the overall parameters showed that physiology of shelled rubber with the PEG 6000 30% coating could be maintain at viability of 99% showing the secondary dormancy of shelled rubber seeds during the storage period of 16 days.

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REFERENCES

AOAC., 1995. Official Methods of Analysis of the Association of Official Analytical Chemistry. 16th Edn., AOAC International, Washington, USA., Pages: 1141.

AOSA., 2002. Seed vigor testing handbook. Contribution No. 32 to the Handbook of Seed Testing, Association of Official Seed Analysts, NE, USA.

APCC., 2005. Standard for virgin coconut oil. Asian and Pacific Coconut Community, Jakarta, Indonesia.

Abdellaoui, R., A. Souid, D. Zayoud and M. Neffati, 2013. Effects of natural long storage duration on seed germination characteristics of *Periploca angustifolia* Labill. Afr. J. Biotechnol., 12: 1760-1768.

Akowuah, J.O., A. Addo and F. Kemausuor, 2012. Influence of storage duration of *Jatropha curcas* seed on oil yield and free fatty acid content. ARPN J. Agric. Biol. Sci., 7: 41-45.

Astoreca, A.L., C.E. Magnoli and A.M. Dalcero, 2010. Ecophysiology of *Aspergillus* section *Nigri* species potential ochratoxin A producers. Toxins, 2: 2593-2605.

Ayranci, E. and M. Sahin, 2008. Interactions of polyethylene glycols with water studied by measurements of density and sound velocity. J. Chem. Thermodyn., 40: 1200-1207.

Bailly, C., A. Benamar, F. Corbineau and D. Come, 2000. Antioxidant systems in sunflower (*Helianthus annuus* L.) seeds as affected by priming. Seed Sci. Res., 10: 35-42.

Bailly, C., J. Leymarie, A. Lehner, S. Rousseau, D. Come and F. Corbineau, 2004. Catalase activity and expression in developing sunflower seeds as related to drying. J. Exp. Bot., 55: 475-483.

Banjongsinsiri, P., 2003. Effect of pectinmethylesterase and calcium infusion on texture and structure of fruits and vegetables. Ph.D. Thesis, Graduate Faculty of the University of Georgia, Athens, Georgia.

Barbedo, C.J., D.D.C. Centeno and R.D.C.L.F. Ribeiro, 2013. Do recalcitrant seeds really exist? Hoehnea, 40: 583-593.

Berchmans, H.J. and S. Hirata, 2008. Biodiesel production from crude *Jatropha curcas* L. seed oil with a high content of free fatty acids. Bioresour. Technol., 99: 1716-1721.

Berjak, P. and N.W. Pammenter, 2004. Recalcitrant Seeds. In: Handbook of Seed Physiology: Applications To Agriculture, Benech-Arnold, R.L. and R.A. Sanchez (Eds.). Haworth Press, New York, pp: 305-345.

Berjak, P. and N.W. Pammenter, 2008. From *Avicennia* to *Zizania*: Seed recalcitrance in perspective. Ann. Bot., 101: 213-228.

Berjak, P. and N.W. Pammenter, 2013. Implications of the lack of desiccation tolerance in recalcitrant seeds. Front. Plant Sci., Vol. 4. 10.3389/fpls.2013.00478

Booth, D.T. and S. Sowa, 2001. Respiration in dormant and non-dormant bitterbrush seeds. J. Arid Environ., 48: 35-39.

Braccini, A.D.L.E., M.S. Reis, M.A. Moreria, C.S. Sediyama and C.A. Scapim, 2000. Biochemical changes associated to soybean seeds osmoconditioning during storage. Pesquisa Agropecuaria Brasileira, 35: 433-447.

Caccere, R., S.P. Teixeira, D.C. Centeno, R.C.L. Figueiredo-Ribeiro and M.R. Braga, 2013. Metabolic and structural changes during early maturation of *Inga vera* seeds are consistent with the lack of a desiccation phase. J. Plant Physiol., 170: 791-800.

Canakci, S. and O. Munzuroglu, 2007. Effects of acetylsalicylic acid on germination, growth and chlorophyll amounts of cucumber (*Cucumis sativus* L.) seeds. Pak. J. Biol. Sci., 10: 2930-2934.

Chao, Y.C., S.K. Su, Y.W. Lin, W.T. Hsu and K.S. Huang, 2012. Interfacial properties of polyethylene glycol/vinyltriethoxysilane (PEG/VTES) copolymers and their application to stain resistance. J. Surfactants. Deterg., 15: 299-305.

Charloq, Z. Lubis, T.H. Siregar, J. Elisa, B.A. Sirait and N.T. Mathius, 2013. PEG 6000 ability test and fungicide efficacy in improving storability of shelled rubber (*Hevea brasilliensis* Muell.Arg) seed. Res. J. Seed Sci., 6: 40-48.

Copeland, L.O. and M.B. McDonald, 2001. Principles of Seed Science and Technology. 4th Edn., Kluwer Academic Publisher, London, Pages: 467.

Corbineau, F., M.A. Picard, J.A. Fougereux, F. Ladonne and D. Come, 2000. Effects of dehydration conditions on desiccation tolerance of developing pea seeds as related to oligosaccharide content and cell membrane properties. Seed Sci. Res., 10: 329-339.

De Alencar, E.R., L.R.D. Faroni, L.A. Peternelli, M.T.C. da Silva and A.R. Costa, 2010. Influence of soybean storage conditions on crude oil quality. Revista Brasileira Engenharia Agricola Ambiental, 14: 303-308.

Deily, K.R. and S.S.H. Rizvi, 1982. Optimization of parameters for packaging of fresh peaches in polymertic films. J. Food Process Eng., 5: 23-41.

Don, R., 2003. ISTA Handbook on Seedling Evaluation. 3rd Edn., International Seed Testing Association, Zurich, Switzerland, ISBN: 3-906549-39-9, Pages: 119.

Enteshari, S. and S. Sharifian, 2012. Influence of salicylic acid on growth and some biochemical parameters in a C_4 plant (*Panicum miliaceum* L.) under saline conditions. Afr. J. Biotechnol., 11: 621-627.

Faria, J.M.R., L.C. Davide, E.A.A. da Silva, A.C. Davide, R.C. Pereira, A.A.M. van Lammeren and H.W.M. Hilhorst, 2006. Physiological and cytological aspects of *Inga vera* subsp. *affinis* embryos during storage. Braz. J. Plant Physiol., 18: 503-513.

Fessel, S.A., R.D. Vieira, M.C.P. da Cruz, R.C. de Paula and M. Panobianco, 2006. Electrical conductivity testing of corn seeds as influenced by temperature and period of storage. Pesquisa Agropecuaria Brasileira, 41: 1551-1559.

Fotouo-M, H., E.S. du Toit and P.J. Robbertse, 2015. Germination and ultrastructural studies of seeds produced by a fast-growing, drought-resistant tree: Implications for its domestication and seed storage. AoB Plants, Vol. 7. 10.1093/aobpla/plv016

Gekas, V., C. Gonzalez, A. Sereno, A. Chiralt and P. Fito, 1998. Mass transfer properties of osmotic solutions. I. Water activity and osmotic pressure. Int. J. Food Properties, 1: 95-112.

Golovina, E.A., H. van As and F.A. Hoekstra, 2010. Membrane chemical stability and seed longevity. Eur. Biophys. J., 39: 657-668.

Gonzalez, M.E., J.A. Jernstedt, D.C. Slaughter and D.M. Barrett, 2010. Influence of cell integrity on textural properties of raw, high pressure and thermally processed onions. J. Food Sci., 75: E409-E416.

Hussain, S., M. Zheng, F. Khan, A. Khaliq and S. Fahad *et al*., 2015. Benefits of rice seed priming are offset permanently by prolonged storage and the storage conditions. Scient. Rep., Vol. 5. 10.1038/srep08101

Ikwuagwu, O.E., I.C. Ononogbu and O.U. Njoku, 2000. Production of biodiesel using rubber [*Hevea brasiliensis* (Kunth. Muell.)] seed oil. Ind. Crops Prod., 12: 57-62.

Konarska, A., 2013. The relationship between the morphology and structure and the quality of fruits of two pear cultivars (*Pyrus communis* L.) during their development and maturation. Scient. World J., Vol. 2013. 10.1155/2013/846796

Kozeko, L.E. and V.M. Troyan, 2000. The relationship between the mitotic activity and moisture content of recalcitrant seeds of *Acer saccharinum* (L.) during maturation, post-maturation drying and germination. Seed Sci. Res., 10: 225-232.

Li, Z., H. Yang, P. Li, J. Liu, J. Wang and Y. Xu, 2013. Fruit biomechanics based on anatomy: A review. Int. Agrophys., 27: 97-106.

Matthews, S. and A.A. Powell, 2006. Electrical conductivity vigour test: Physiological basis and use. ISTA News Bull., 131: 32-35.

Navaey, H.N., M.Y. Hzari, R.A.N. Seraji and H. Eslami, 2014. Germination reduce in Borage (*Borago officinalis* L.) seed under seed deteriorating conditions. Int. J. Farming Allied Sci., 3: 358-361.

Osawa, C.C., L.A.G. Goncalves and S. Ragazzi, 2007. Correlation between free fatty acids of vegetable oils evaluated by rapid tests and by the official method. J. Food Compos. Anal., 20: 523-528.

Pammenter, N.W. and P. Berjak, 2000. Aspects of recalcitrant seed physiology. Revista Brasileira Fisiologia Vegetal, 12: 56-69.

Pammenter, N.W. and P. Berjak, 2014. Physiology of desiccation-sensitive (recalcitrant) seeds and the implications for cryopreservation. Int. J. Plant Sci., 175: 21-28.

Pardo, E., V. Sanchis, A.J. Ramos and S. Marin, 2006. Non-specificity of nutritional substrate for Ochratoxin A production by isolates of *Aspergillus ochraceus*. Food Microbiol., 23: 351-358.

Pukacka, A. and E. Ratajczak, 2006. Antioxidative response of ascorbate-glutathione pathway enzymes and metabolites to desiccation of recalcitrant *Acer saccharinum* seeds. J. Plant Physiol., 163: 1259-1266.

Ramos, K.M.O., J.M. Matos, R.C. Martins and I.S. Martins, 2012. Electrical conductivity testing as applied to the assessment of freshly collected *Kielmeyera coriacea* Mart. seeds. ISRN Agron., Vol. 2012. 10.5402/2012/378139.

Rohandi, A. and N. Widyani, 2011. [Analysis of physiological and biochemical of tengkawang seeds during dessication]. Jurnal Penelitian Hutan Tanaman, 8: 31-40, (In Indonesian).

Salah, S.M., G. Yajing, C. Dongdong, L. Jie and N. Aamir *et al*., 2015. Seed priming with polyethylene glycol regulating the physiological and molecular mechanism in rice (*Oryza sativa* L.) under nano-ZnO stress. Sci. Rep., Vol. 5. 10.1038/srep14278

Schuck, P., A. Dolivet and R. Jeantet, 2012. Determination of the Ash Content. In: Analytical Methods for Food and Dairy Powders, Schuck, P., R. Jeantet and A. Dolivet (Eds.). John Wiley and Sons, New York, USA., ISBN-13: 9781118307427.

Setyowati, N., 2009. The effect of seed maturity, temperature and storage period on vigor of *Picrasma javanica* Bl. seedling. Biodiversitas, 10: 49-53.

Shokib, A., P. Gumanti and M.R. Rachimoellah, 2010. Biodiesel production from rubber seed oil by supercritical methanol method. IPTEK: J. Technol. Sci., 21: 223-229.

Steel, R.G.D. and J.H. Torrie, 1995. Principles and Procedures of Statistics. McGraw-Hill Book Co. Inc., New York, USA.

Sung, J.M. and K.Y. Chiu, 2001. Solid matrix priming can partially reverse the deterioration of sweet corn seeds induced by 2,2'-azobis (2-amidinopropane) hydrochloride generated free radicals. Seed Sci. Technol., 29: 287-298.

Tabin, T. and K. Shrivastava, 2014. Factors affecting seed germination and establishment of critically endangered *Aquilaria malaccensis* (Thymelaeaceae). Asian J. Plant Sci. Res., 4: 41-46.

Tatipata, A., 2009. Effect of seed moisture content packaging and storage period on mitochondria inner membrane of soybean seed. J. Agric. Technol., 5: 51-64.

Tweddle, J.C., J.B. Dickie, C.C. Baskin and J.M. Baskin, 2003. Ecological aspects of seed desiccation sensitivity. J. Ecol., 91: 294-304.

Varier, A., A.K. Vari and M. Dadlani, 2010. The subcellular basis of seed priming. Curr. Sci., 99: 450-456.

Vieira, R.D., A.S. Neto, S.R.M. de Bittencourt and M. Panobianco, 2004. Electrical conductivity of the seed soaking solution and soybean seedling emergence. Scientia Agricola, 61: 164-168.

Wu, X.J., 2002. Correlation of physio-chemical characteristics in the seed coat and canning quality in different dark red kidney bean (*Phaseolus vulgaris* L.) cultivars. M.Sc. Thesis, The Graduate School University of Wisconsin-Stout, Wisconsin, USA.