

# Seed germination characteristics in different storage time of *Gmelina arborea* treated with ultrafine bubbles priming

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**Abstract.** Siregar IZ, Muharam KF, Purwanto YA, Sudrajat DJ. 2020. Seed germination characteristics in different storage time of *Gmelina arborea* treated with ultrafine bubbles priming. *Biodiversitas* 21: 4558-4564. *Gmelina* (*Gmelina arborea*) seed collected from tropical Indonesian forest experience seed deterioration during storage which is relatively faster than the *gmelina* seeds originating from temperate regions, such as India and Myanmar. Various treatments have been made to improve the seed viability and vigor after storage through various invigoration techniques. However, the utilization of promising novel technology such as ultrafine bubbles (UFB) has not been evaluated yet. The objective of this study was to determine the effect of seed priming using UFB, polyethylene glycol (PEG), and gibberellic acid (GA<sub>3</sub>) on the viability and vigor of *gmelina* seeds that have been stored for one and two years. This study was conducted by employing Completely Randomized Design (CRD) using three replicates. The study showed priming treatment had significant effect and formed average germination capacity of 80% for the seed stored for 1 year. On the other hand, the priming treatment using PEG-0.8 MPa was the best treatment for germination capacity of 74.67% on 2 years stored seeds. UFB had significant effect on germination capacity, germination rate, growth uniformity, and germination value. This result was expected due to reactive oxygen species (ROS) produced by micro-nano bubbles that could increase physiological activity of the seed cells.

**Keywords:** *Gmelina arborea*, seed storage, priming

## INTRODUCTION

*Gmelina* (*Gmelina arborea* Roxb.) is being cultivated more and more in industrial plantation forest and community forest in Indonesia as it is a fast-growing tree species capable of producing crops in just 5 to 6 years rotation, under intensive silviculture (Kosasih and Danu 2013). *Gmelina* can easily be propagated, but the availability of seed for producing adequate quantities of seedlings for large-scale plantation activities is a major limitation. In India as the original distribution site of the *gmelina*, the storage of seeds is still problematic as seeds lose their viability over a short storage period (Prakash 1991; Naithani et al. 2006). The deterioration rate of seed during storage is faster on the seed from plants growing in moist tropical rain forests than plants in temperate regions (Sudrajat et al. 2017) and this also happened to the *gmelina* seeds. Seed deterioration would be occurred as long as storage caused by physical, physiological, and biochemical changes such as loss of membrane integrity, protein synthesis, reduced energy metabolism, DNA degradation, and impairment of RNA in the seed affected on declining of seed viability and vigor (McDonal, 1999; Shaban, 2013). One of the efforts to increase seeds viability and vigor that have suffered from deterioration is the invigoration treatments, such as hydro-priming or osmo-priming.

Priming techniques can improve seed physiological and biochemical conditions and increase the potential of seeds

to germinate (Khan et al. 1992). This technique is very important and useful to improve the seed viability and vigor in procuring high-quality seeds and genetic conservation attempts. The improvement in seed germination depends on several factors, including plant species, immersion period, temperature, vigor, and seed storage condition and period (Mubshar et al. 2006). Priming treatment can be performed using osmotic solutions or essential plant hormones. The osmotic solution commonly used in physiological seed improvement is polyethylene glycol (PEG) that can play a role as regulation of seed water accessibility through imbibition or desorption. Syamsuddin (1998) stated that osmo-conditioning using PEG 6000-0.8 MPa had a significant effect on the germination rate and vigor of *gmelina* seeds. Rouhi et al. (2010) reported that PEG-6000 priming treatment can increase germination capacity, uniformity of growth, weight of normal seedlings, mean germination time, vigor index, hypocotyl length, and epicotyl length. Gibberellic acid (GA<sub>3</sub>) is a hormone produced by plants, but in the low vigor seeds, it is often very limited in number to support the germination process, so that additional immersion of the external gibberellins' hormone is expected to stimulate cell division and increase germination. Asra (2014) reported that the maximum concentration to stimulate kalopo (*Calopogonium caeruleum*) seed germination using GA<sub>3</sub> immersion was 500 ppm.

Oxygen concentrations in tissues play a role in regulating biochemical reactions (Wagner 2008). In recent years, many studies have shown the potential and benefits of dissolved oxygen in water. Micro and nanobubbles are renewable technology contained in water and can increase plant growth (Liu et al. 2014). Ultrafine bubbles (UFB) are minuscule bubbles with diameters ranging between micro and nanoscales. Microbubbles have a dimension ranging from  $10^{-4}$  to  $10^{-6}$   $\mu$ , whereas nanobubbles are smaller than  $10^{-6}$  (Uchida et al. 2011). Nearly all-micro bubbles can subsist in water for only about 10 minutes, whereas nanobubbles are relatively stable and can last for several days in water. Liu et al. (2013) reported that the germination percentage of wheat seeds increased about 15-25% after water immersion treatment containing nanobubbles with a certain dissolved oxygen concentration. Some studies of the positive effect of UFB water on enhancing germination capacity reported by Purwanto et al. (2019) on soybean seeds and Fata et al. (2020) on white jabon seeds. The utilization of UFB for forest plant seeds is still very limited, therefore, the main objectives of this study were to determine the effectiveness of UFB for improving the viability and vigor of stored *gmelina* seeds and its comparison with other priming techniques, such as PEG and  $GA_3$  treatments.

## MATERIALS AND METHODS

### Materials

Materials used in this study were *gmelina* seeds collected from Nagrak Research Station, Bogor. The weather (temperature and rainfall) data of Nagrak Research Station based on the data collected in 2016 and 2017 is presented in Figure 1. The location of the *gmelina* stands in Nagrak, Bogor has an average temperature of 27.8 °C and a total rainfall of 2156.83 mm per year. *Gmelina* is a plant that blooms every year thus it always produces seeds. Alrasyid and Widiarti (1992) stated that *gmelina* will grow optimally at an altitude of 0-800 m asl, with temperatures ranging from 21°-28°C and annual rainfall from 1778 mm to 2286 mm. Subject to this information, the location of *gmelina* stand is suitable for plant development and reproduction process. The seeds were stored in an air conditioner room (temperature 18-20°C, humidity 40-60%). The other materials are ultrafine bubbles by means of non-injection (8.5 ppm) and injection treatment (20.56 ppm), PEG Solution 6000-0.4 MPa and-0.8 MPa,  $GA_3$  250 ppm and 500 ppm, aquadest, fungicide with active ingredients mancozeb, and sterile sand media. Meanwhile, the instrument used to perform the study included ultrafine bubbles generator (UltrafineGaLF tipe FZ1N-10), Petri dishes, test tubes, autoclaves, oven, desiccators, scales, label paper, sand filters, germination tray, and spray bottle.

### Experimental design

The experimental design used in this study was a Completely Randomized Design (CRD) with 7 priming treatments. The priming treatment consists of: seed control (P1), PEG 6000 0.4 MPa (P2), PEG 6000 1.2 MPa (P3),

$GA_3$  250 ppm (P4),  $GA_3$  500 ppm (P5), non-injection ultrafine bubbles (P6), and injection ultrafine bubbles (P7). The priming treatment was carried out on 2 seed lots with different storage periods, i.e. 1-year and 2-year storages, analyzed by CRD separately. Each treatment was carried out with three replicates and each replicate consisted of 50 seeds.

Linear model of the experimental design used was as follows:

$$Y_{ijk} = \mu + \alpha_i + \varepsilon_{ij}$$

Where:

$Y_{ijk}$  : observation value on the  $i^{\text{th}}$  invigoration treatment and  $j^{\text{th}}$  replicate

$\mu$  : mean of the population

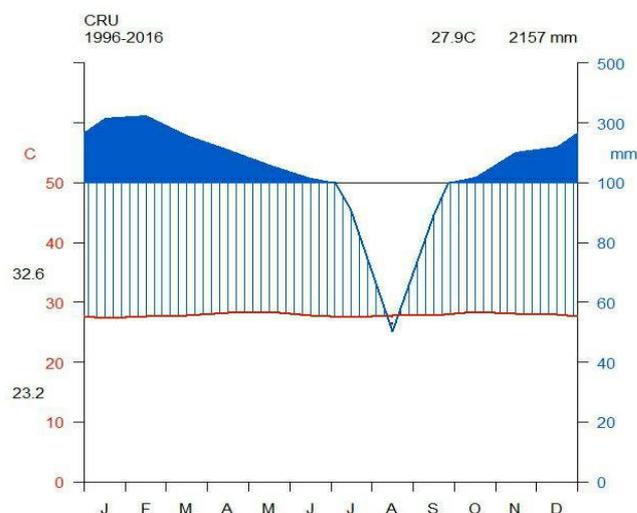
$\alpha_i$  :  $i^{\text{th}}$  invigoration effect

$\varepsilon_{ij}$  : error variance in normal distribution

The parameters used to determine the effect of priming on the *gmelina* seeds viability and vigor were germination capacity (GC) (Sudrajat et al. 2017), T50 (time which is needed to reach 50% of normal seedlings from the maximum normal seedlings) (Fata et al., 2020), germination rate (GR), germination value (GV), (Djavanshir and Pourbeik 1976), growth uniformity (GU), vigor index (VI) (Bhattacharya et al. 1991), radicle length, hypocotyl length, and normal seedling biomass (Sudrajat, 2016). The hypocotyl and radicle length was recorded at the end of the germination test. For biomass measurements, all normal seedlings were harvested and dried in a drying oven at 70°C for 48 h and weighed to  $\pm 0.0001$  g.

### Data analysis

The observation data were analyzed with SAS® software version 9.1 employing ANOVA (analysis of variance) program. If the ANOVA shows a significant effect then the analysis continues with Duncan's Multiple Range Test (DMRT) at 5% level to determine the differences between priming treatments.



**Figure 1.** Temperature and rainfall of the location of seed collection at Nagrak Research Station, Bogor (Source: Climatic Research Unit, <http://www.cru.uea.ac.uk>)

## RESULTS AND DISCUSSION

### Effect of priming treatment based on different storage periods on seed viability and vigor

Analysis of variance showed that priming treatments using PEG, GA<sub>3</sub> and UFB on the gmelina seeds stored for 2 years had a significant effect only on hypocotyl length, while the treatments on the gmelina seeds stored for 1 year significantly affected on the germination capacity (GC), germination rate (GR), germination value (GV), growth uniformity (GU), and hypocotyl length (Table 1).

The priming treatments using PEG, GA<sub>3</sub>, and UFB were only effective on the gmelina seed that was stored for 1 year. The gmelina seeds stored for 2 years are thought to have suffered greater damage than seeds stored for 1 year. The length of storage periods of seeds can cause a decrease in seed quality, both physically, physiologically, and chemically resulting in decreased viability and vigor of seeds (Mustika et al. 2014). There is no significant effect of the priming treatments on germination of very low seed viability and vigor also reported by Maisura et al. (2016) on *Oryza sativa* seeds, and Fata et al. (2020) on *Neolamarckia cadamba* seeds. Nurmauli and Nurmiaty (2010) reported that the priming treatment was more effective in soybean seeds with high initial germination than the low ones. Meanwhile, Wahyuni (2011) showed that invigoration treatment of medium quality rice seeds could increase seed vigor higher than low-quality rice seeds.

### Seed viability and vigor based on various priming treatments on different storage periods

Principally, seed germination illustrated in three stages, including the imbibition process, begins the metabolic process followed by the development of radicles from the seeds. Germination is a measure of seed viability, i.e. a measure of how many seeds are alive and could develop into plants that will reproduce themselves, given the appropriate conditions (Sudrajat et al. 2017). Vigor is the sum of those properties of the seed that determine the potential level of activity and performance of the seed during germination and seedling emergence (Perry 1980; ISTA 1995). In this study, the seed viability and vigor were identified by which can be identified by some germination parameters presented in Figure 2.

Figure 2.a. showed that priming treatment with UFB injection produces the highest germination capacity on 1-year stored seeds, which was 80%. This is in line with Liu et al. (2014) which conducted the research on wheat seeds, micro-nano bubbles treatment can increase the germination capacity of wheat seeds by 66% while for the control by 43%. UFB injection is water containing micro-nano bubbles with dissolved oxygen of 20.56 mg/L. According to Liu et al. (2014), water containing micro-nano bubbles can form Reactive Oxygen Species (ROS) or free radicals. ROS or free radicals have a crucial role in the germination process (Liu et al. 2017). Essentially, free radicals can increase the physiological activity of living things, especially plants, but in high figures might cause damage to

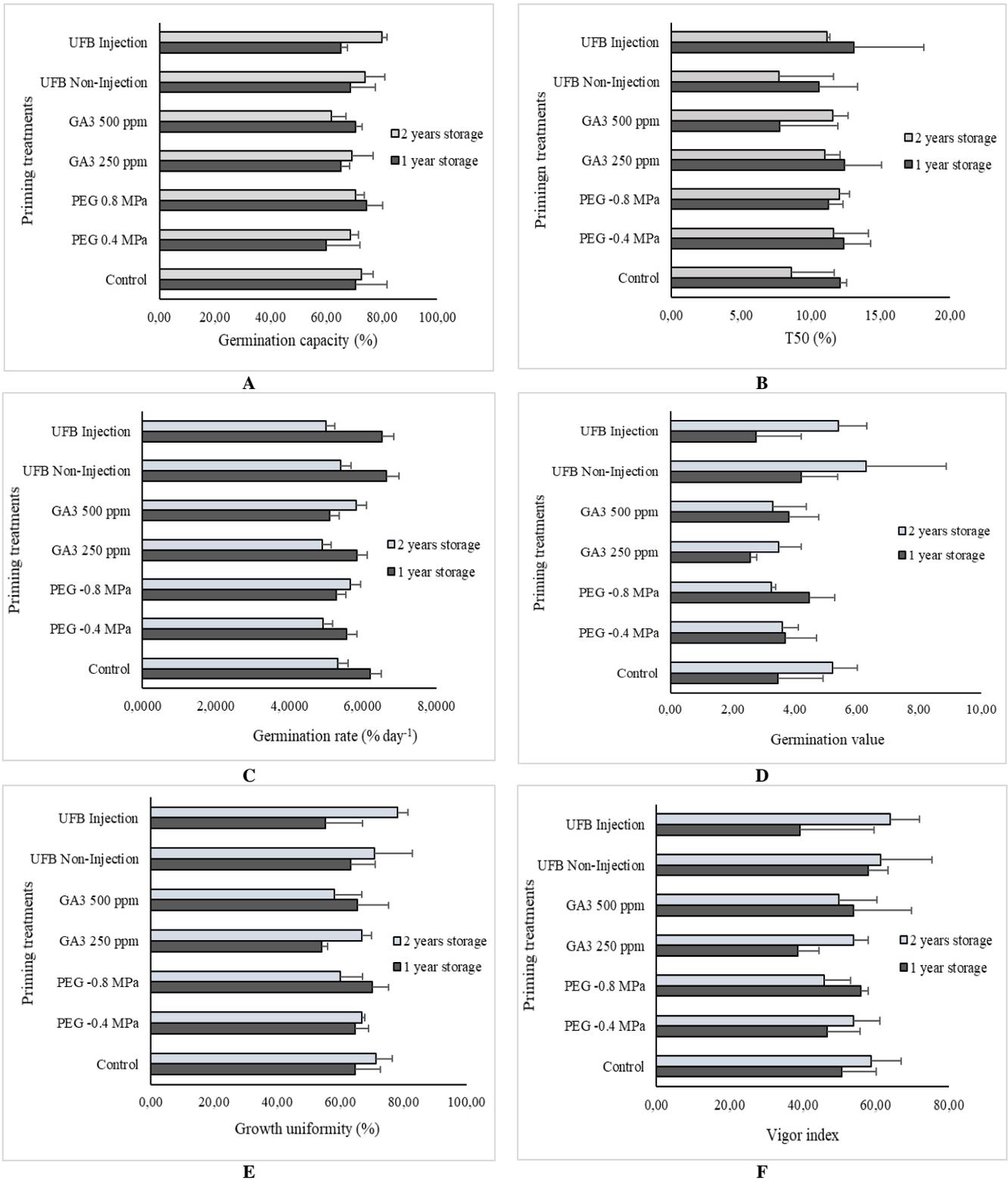
the plant (Liu et al. 2014). The gmelina seeds with 2 years storage period, obtained germination capacity ranging from 58%-80%. The priming treatments on the 2 years stored gmelina seeds have not affected the seed germination. Nevertheless, the best germination capacity was obtained by priming treatment of PEG-6000 solution with a concentration of 0.8 MPa. Seed storage period can lead to seeds properties deterioration both in terms of seed viability and vigor. PEG-6000 solution influences seed imbibition and hydration in which treatment on high PEG concentration can increase germination capacity of 2 years stored gmelina seeds. Figure 2.b. shows that priming treatment has no significant effect on T50. T50 is time which is needed to reach 50% of normal seedlings from the maximum normal seedlings. Unlike the other parameters, lower T50 value indicates faster seeds germination. However, the implementation of GA<sub>3</sub> 500 ppm and UFB non-injection treatment stimulates the fast emergence of seedlings at 7.88 days and 7.32 days. Seed germination can be influenced by seed metabolism, especially seed vigor. This priming treatment by means of immersion technique stimulates seed imbibition to be able to quickly activate the required enzymes germination process.

The ability of seeds to grow and develop into normal and resistant seedlings under suboptimum conditions demonstrates sublime seed vigor. Seeds with high germination rate associated with decent seed viability. Germination rate is also a reflection of the seed individual vigor connected with time (Copeland and McDonald 2001). Germination rate was measured based on the percentage of normal germination every 24 hours (% day<sup>-1</sup>). Based on Figure 2.C., UFB priming treatment was significantly affected the growth rate of gmelina seeds, both on non-injection and injection UFB treatment. Similar to a study by Liu et al. (2017), that the density of micro-nano bubbles affects the germination percentage at the end of the observation time and accelerates the germination of spinach and wheat seeds. The low density of micro-nano bubbles does not imply negative effects on the observed seeds but manages to increase its germination properties.

**Table 1.** Recapitulation of the analysis of variance of various priming treatments based on the storage period to the observed germination parameters

Parameters	Storage period	
	2 years	1 year
GC (%)	0.3510 <sup>ns</sup>	0.0228 <sup>*</sup>
T50 (days)	0.4633 <sup>ns</sup>	0.1309 <sup>ns</sup>
GR (% day <sup>-1</sup> )	0.6179 <sup>ns</sup>	0.0277 <sup>**</sup>
GV	0.3317 <sup>ns</sup>	0.0339 <sup>*</sup>
GU (%)	0.1926 <sup>ns</sup>	0.0373 <sup>**</sup>
Vigor index	0.2756 <sup>ns</sup>	0.2449 <sup>ns</sup>
Radicle length (mm)	0.1418 <sup>ns</sup>	0.5213 <sup>ns</sup>
Hypocotyl length (mm)	0.0006 <sup>*</sup>	0.0006 <sup>*</sup>
Normal seedling biomass (g)	0.7010 <sup>ns</sup>	0.4505 <sup>ns</sup>

Note: \*\* significant at 1% level, \* significant at 5% level, ns: no significant.



**Figure 2.** Seed viability parameters: A. Germination percentage, B. T50, C. Germination rate, D. Germination value, E. Growth uniformity, F. Vigor index based on different storage periods on various priming treatments

Figure 2.D. confirms the total germination values obtained during 28 days of observation on the *gmelina* germination process. Germination value is the multiplication result between PV (Peak Value) or

germination peak value with MDG (Mean Daily Germination). The amount of germination is determined from the peak value of germination with the highest percentage of germination. This peak value is also

commonly used to determine the seed germination speed (Djavanshir and Pourbeik 1976). Based on the results of variance testing, the value of germination seed significantly affected 1-year stored gmelina seeds, while the 2-years stored gmelina seeds expressed no significant effect after subjected to the priming treatment. The best treatment was the non-injection UFB treatment with a germination value of 6.28. The seed germination value is a combination of speed and germination percentage of the seed. The germination value is associated with the seed longevity or resistance. Non-injection UFB treatment prolonged seed viability by producing energy for seeds from ROS.

Seedling growth uniformity, Figure 2.e., is one measure of seed vigor based on normal seedlings that grow between the first count and the final count. This can indicate normal seedlings that are classified as strong. Based on the data, the priming treatment with a PEG-0.4 MPa solution showed uniform data with an equally low interval. The UFB injection treatment produced varied and highest data with an average of 78%. UFB treatment as previously explained, can increase germination. According to Ushikubo et al. (2008), wheat seeds treated with micro-nano bubbles water immersion treatment exhibits faster cytoplasm flow. This mechanism is responsible for increasing seed vigor and growth uniformity. High growth uniformity indicates high storability of seed as well. Thus, the seeds with fast germination rate and high growth uniformity would likely possess high levels of vigor.

Vigor index calculated by seedling length and percent germination and Figure 2.F. shows a graph of the vigor index in several priming treatments. Seed storage process can cause seed quality deterioration followed by water content reduction. Priming treatment is an attempt to intensify the seed properties. Seeds with high vigor portray their ability to grow into normal seedlings and can survive under suboptimum conditions. Normal seedlings that emerge and grow well at the first cycle or the time after the planting also show good seed vigor. In this study, priming treatment did not have a significant effect on the gmelina seed vigor index (Tabel 1). Figure 2.f. shows that the best treatment for the 2-years and 1-year storage periods gmelina seeds in was non-injection UFB and injection

UFB, respectively. High seed vigor is characterized by seed storage longevity, pests, and disease resistance, possessed fast and uniformity growth traits, and able to produce normal mature plants with decent reproductive properties in a sub-optimal growing environment (Finch-Savage and Bassel 2016; Rao et al. 2017).

#### Radicle length, hypocotyl length, and normal seedling biomass

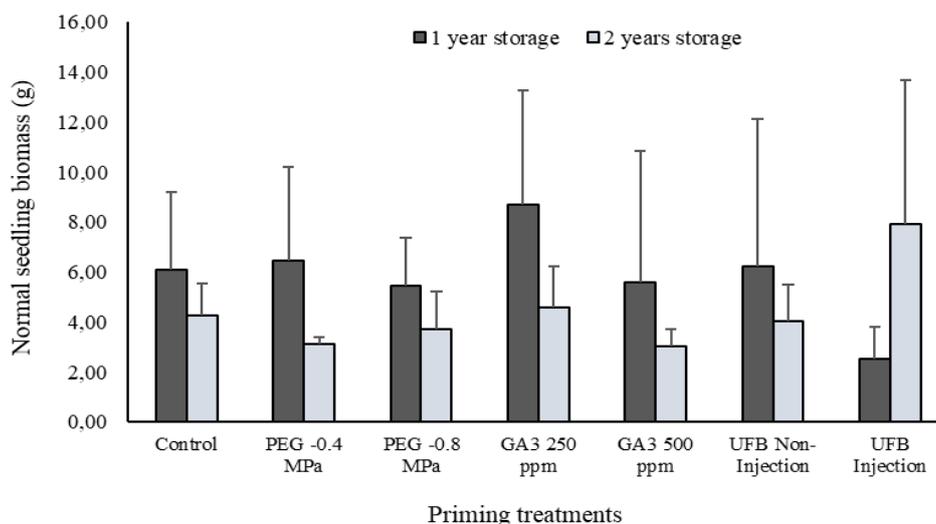
Besides germination parameters, the standard of a seed eminence can also be seen from the radicle and hypocotyl length measured at the end of the observation (Sontani 2001). Based on Tabel 2, priming treatment using GA<sub>3</sub> has a significant effect on hypocotyl length. The longest hypocotyl length is at the priming treatment of GA<sub>3</sub> 250 ppm and GA<sub>3</sub> 500 ppm with lengths of 3.88 cm and 3.82 cm, respectively. GA<sub>3</sub> is one of plant hormones that take part in seedling growth. Seed immersion using GA<sub>3</sub> during priming process increases seed availability to stimulate seedling growth. In line with this result, Gurung et al. (2014) stated that the length of seedling stems treated with GA<sub>3</sub> 500 ppm and 250 ppm formed the longest hypocotyl. Pancaningtyas and Santoso (2014) implied the amylase enzyme in plants will be formed and convert starch to sugar as an energy source for growth due to optimum gibberellins immersion. Moreover, gibberellin in plants stimulates stem elongation, while auxin is inhibiting root growth at concentrations above 10<sup>-9</sup> M (Maemunah and Adelina 2009). The ability of gibberellins to affect the hypocotyl length can also be suspected from seeds water absorption activity (imbibition) is effective during the immersion. The shortest hypocotyl length was the control sample with a length of 1.79 cm.

Normal seedling biomass indicates abundant nutrient content reserves in the plant. According to Moshatati and Gharineh (2016), seeds weight and seedling length can be determined through their biomass or dry weight. In addition, seedling biomass indicates the amount of nutrient supply inside the plant. High seeds vigor allow seeds to have enough energy to germinate and supply nutrient stock during the germination process (Timotiwi et al. 2017).

**Table 2.** Effect of invigoration treatment on radicle length (mean ± SD) and hypocotyl length (mean ± SD) of different storage periods

Treatment	Radicle length(cm)		Hypocotyl length(cm)	
	1 year stored	2 years stored	1 year stored	2 years stored
Control	7.36 ± 0.64	8.00 ± 1.45	1.79 ± 0.05 d	2.32 ± 0.22 c
PEG-0.4 MPa	7.29 ± 0.80	6.88 ± 1.00	2.68 ± 0.36 bc	2.71 ± 0.25 bc
PEG-0.8 MPa	5.25 ± 1.19	5.77 ± 1.80	2.56 ± 0.63 bc	2.61 ± 0.20 bc
GA3 250 ppm	7.10 ± 2.40	6.29 ± 1.28	2.92 ± 0.20 b	3.88 ± 0.10 a
GA3 500 ppm	6.90 ± 1.54	7.58 ± 2.07	3.82 ± 0.69 a	3.82 ± 0.23 a
Non-Injection UFB	8.10 ± 0.98	6.99 ± 1.72	2.93 ± 0.21 b	3.05 ± 0.79 b
Injection UFB	8.55 ± 0.48	6.06 ± 0.47	2.17 ± 0.27 c	2.68 ± 0.28 bc

Note: The data shown are mean ± standard deviation of replicates; Different letters (a, b, c, d, and bc) in the same column indicate significant differences at  $P \leq 0.05$  between treatment



**Figure 3.** Normal seedling biomass of *gmelina* based on various priming treatments on different storage periods

Figure 3 shows the normal seedling biomass was not affected by priming treatment. Based on these data, it appears that GA<sub>3</sub> 250 ppm was the best treatment with average normal seedling biomass of 8.69 g. In line with the previous data, the longest hypocotyl length of *gmelina* sprouts has resulted from GA<sub>3</sub> 250 ppm treatment. The high value of the normal seedling biomass presumably due to the photosynthesis absorbed by plants. Seed storage process tends to reduce seed viability and vigor (El-Refaey and El-Dengawy 2005). Priming technique with Ultra Fine Bubbles (UFB) and GA<sub>3</sub> 250 ppm provide the best results for several germination parameters. The 1-year stored *gmelina* seeds expressed the best germination traits by means of UFB injection treatment with germination capacity of 80%, 6.65% day<sup>-1</sup> germination rate, 6.29 germination value, and 78% growth uniformity. Meanwhile various applied priming treatment on the 2-years stored *gmelina* seeds did not show any significant effect on the seed viability and vigor. GA<sub>3</sub> treatment was solely affected by seedling growth properties, such as hypocotyl length and total normal seedling biomass.

In conclusion, *gmelina* is considered as one of the important species for promoting plantation forests in private lands since the wood industry is also available to absorb the timber as raw materials. Quality seeds that are productive, adaptive and harbor certain level of sufficient genetic diversity are needed to promote the tree planting programs for many purposes, particularly for ecosystem restoration and climate mitigation. Access of the technology application to farmers is also necessary to be considered through the provision of cost-effective UFB for treating the stored *gmelina* seeds after one year. The research however still needs further improvement to enable the monitoring of seedling quality resulted from UFB and PEG treatments.

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