INTRODUCTION

Oil palm (*Elaeis guineensis* L. Jacq) is a perennial plant that generates the highest vegetable oil productivity (Corley & Tinker, 2015; Lubis & Lubis, 2018) with the finest effectivity on production cost compared to other oil-producing plant species (Singh, 2014). In Indonesia, the planting area of the species has grown rapidly in the last few decades. Direktorat Jenderal Perkebunan (2020) expected that by 2021, Indonesia would have 15.1 million hectares of oil palm area, more than 100 times the area when the recording of oil palm area was first carried out in Indonesia in 1970, at 133,298 hectares. The increasing size of oil palm plantations in Indonesia is driven by the high demand for crude palm oil (CPO) as the world’s population increased, as well as the need for renewable energy, replacing fossil fuels that could run out at any time (Helwani, Saputra, Fatra, & Herman, 2016; Masykur, 2013). Directorate of Energy, Mineral and Mining Resources of the Republic of Indonesia (Direktorat Sumber Daya Energi, Mineral dan Pertambangan, 2015) noted that oil palm has great potential as raw material for various biofuels, i.e., biodiesel, biogas, bio pellets, bio briquettes, methane gas, and biomass power plants.

The increment of oil palm plantation areas in Indonesia increases the demand for oil palm planting materials. Using data series from 1998 to 2009, Liwang, Daryanto, Gumbira-Said, & Nuryartono (2011) stated that at the national level, there was a strong positive correlation between the number of oil palm seed sales in a certain year.
and the increase in oil palm seed sales in certain areas in the following calendar year. They predicted that the national demand for oil palm germinated seeds would be around 150 million seeds per year, indicating the high market for legitimate plant materials at the user level.

In several cases, hindrance in supplying oil palm germinated seeds is low seed germination percentage due to physical dormancy in the form of a thick shell, known as endocarp (Farhana, Ilyas, & Budiman, 2013; Green et al., 2013), where the method of breaking oil palm seed dormancy at industrial level is the dry heat method which was introduced in 1953 and first used commercially in Malaysia six years later (Herrera, Alizaga, & Guevara, 1998). Although this method is still applied today in many countries which generate oil palm germinated seeds, Murugesan, Ravichandran, & Shareef (2015) stated that the dry heat method has several disadvantages, such as extra time is required for a decent percentage of germination to be achieved, while Ravichandran, Murugesan, Naveen Kumar, Mathur, & Ramajayam (2016) added that interruption during a dry-heat process may lower rate of normal germinated seeds.

A method currently being developed to break seed physical dormancy is using fine bubble water, which has been shown to positively affect white jabon (Anthocephalus cadamba) (Fata, Supriyanto, Rustam, & Sudrajat, 2020) and sandalwood seeds (Maia, Qadir, Widajati, & Purwanto, 2021). Therefore, fine bubble water application should be tested during oil palm seed germination. In addition, the research also aims to investigate the effects of different soaking duration and fiber plug removal on seed moisture content (SMC) and germination percentage of oil palm seeds.

MATERIALS AND METHODS

The experiment was conducted from June to December 2020 in two locations, i.e., the seed processing unit of the Indonesian Oil Palm Research Institute (IOPRI) in Medan, North Sumatra, and the Material Analysis Laboratory, Department of Physics, Faculty of Mathematics and Science, IPB University, West Java, Indonesia.

As five replications, dura-type seeds derived from five control-pollinated bunches were treated with the dry heat method as described by Tabi, Ngando Ebongue, Ntsomboh, & Youmbi (2017) to break the dormancy of the seeds. The dry heat method was started by soaking the seeds in water (first soaking) for 7 days which water was replaced daily, followed by air drying for approximately 8 hours until the SMC of about 17-18%. Seeds were then placed in a heat chamber with an air temperature of 39 ± 1°C for 60 days. Afterward, seeds from each bunch were divided into two equal sets. In the first set, the seeds were left with intact fiber plugs, while fiber plugs in the second set were removed using cutter knives (as the first factor). Both sets were then divided into six randomized groups in which each group was treated with specific soaking treatment as the second factor, i.e., tap water for 1 day (TW1), 2 days (TW2), 3 days (TW3), fine bubble water for 1 day (FB1), 2 days (FB2), and 3 days (FB3). The treatments were replicated five times, and 100 to 150 seeds were used for each experimental unit, depending on the number of seeds produced per bunch.

SMC was determined using an oven at a constant low temperature of 103 ± 2°C for 48 ± 3 h (Arif et al., 2020). Fine bubble water was generated using Fit & Grow fine bubble generator type NG3. The particle size analysis of FB water was carried out at the Material Analysis Laboratory of IPB University using a VascoTM nanoparticle size analyzer (Corduan Technologies).

After the second soaking of the dry heat method, the seeds were placed in a germination room with a temperature of 32-34°C. Evaluation of germination began on the seventh day after germination (DAG) and was observed daily for germination occurrences. Seeds were considered germinated when 0.5 mm of plumule and radicle emerged from the seed. Experimental units were inspected until 49 days in the germination room, where ungerminated seeds were considered dead after that day.

Data gained from the experiment were analyzed using SPSS ver. 16 (IBM). The means for treatment were compared using the Duncan multiple range test at a probability of P < 0.05.

RESULTS AND DISCUSSION

Bubble Size

Based on the bubble size, Liu et al. (2016) and Uchida, Nishikawa, Sakurai, Asano, & Noda (2018) reported that fine bubbles are those with a diameter of fewer than 100 micrometers, consisting of microbubbles with sizes between 1-100 micrometers, and nano or ultrafine bubbles with dimensions less than 1 micrometers. The bubbling water in this experiment was generated from tap
water flown into a bubble generator (Fit & Grow type NG3). The particle size analysis showed that the bubbles’ average diameter was 405.47 nm, and 50% of the sample volume (Dv50) had a maximum bubble diameter of 537.17 nm, which indicated that the bubbles were categorized as ultrafine bubbles (Fig. 1). However, some of the bubbles had a diameter of >1000 nm, and 10% of the sample volume had a bubble size of >1,349.32 nm (Dv90), indicating that a small part of the generated bubbles falls into the microbubbles category.

Seed Physiological Parameters

The use of fine bubble water in the dry heat method for breaking the dormancy of oil palm seeds improved germination rates either with or without fiber plugs (Fig. 2c and 2d). Albeit all treatments started to germinate at 21 DAG, seeds that were treated with fine bubble water had a higher germination percentage on day 22 (4.67% and 4.40% respectively for intact and fiber plug removed seeds, respectively) compared to those which were soaked in tap water (1.87% and 0.33%). However, the treatments did not provide significant differences in total germination percentage, where tap water delivered 76.2 and 61.6% for intact and fiber plug-removed seeds, while fine bubble treatments generated 71.1% and 68.1%, respectively.

Germination peaks which were shown by the fine bubble water treatments (Fig. 2c and 2d), occurred 8 days faster (26 DAG) than those in the tap water treatments (Fig. 2a and 2b), which took place on approximately 34 DAG. The occurrence indicated that using fine bubble water during the second seed soaking in the dry heat treatment process may induce faster germination of oil palm seed.

A commonly utilized method to break oil palm seed dormancy is the dry heat method (Cui, Lamade, & Tcherkez, 2020) which requires more than 100 days for some seeds to germinate (Green et al., 2013; Ravichandran, Murugesan, Naveen Kumar, Mathur, & Ramajayam, 2016). Because of that, the method is considered laborious, expensive, and time-consuming (Cui, Lamade, & Tcherkez, 2020). Due to that reason, the ability of the fine bubble water treatment to induce higher percentages of germinated seeds at an early stage of germination indicates that the treatments have the potential to shorten the seed germination process and generate lower seed production costs.

Based on the vigor index, no significant differences occurred between tap water and fine bubble water treatments, where applications on intact seeds showed that 50% of the tested seeds were germinated on day 34 (Fig. 2a and 2c), while on treatments where fiber plugs were removed, the occurrences happened on day 41 and 38 (Fig. 2b and 2d), respectively. Besides indicating that fine bubble treatment during second seed soaking in the dry heat treatment process was not effective enough to hasten the oil palm seed vigor index, the phenomenon also showed that utilization of intact seeds provided a better vigor index than that on seeds in which fiber plugs were removed. This occurrence is contrary to the findings of Chanprasert, Myint, Srikul, & Wongsri (2012), which generated a better seed vigor index of operculum-removed seeds than that of intact ones. This might be due to the removed components of the two types of research were different where Chanprasert, Myint, Srikul, & Wongsri (2012) removed not only the fiber plugs of the seeds, as was conducted for this study, but removed the whole operculum (fiber plug, testa, and endosperm) for the embryo to be exposed.

Fig. 2 also shows that fine bubble water treatments during second seed soaking generate higher early germination. On days 22 and 24, tap water treatments only produce 1% of germination, compared to those fine bubble water treatments, which comprised approximately 5% of germination. The occurrence where fine bubble water treatment promotes early germination percentages were also shown by Sritontip, Dechthummarong, Thonglek, Khaosumain, & Sritontip (2019) on Chinese celery (Apium graveolens L.) and sweet corn (Zea mays L.) and Chanchula, Pivsa-Art, Wattanawikram, & Porjai (2019) on sunflower seeds (Helianthus annuus) seeds.
Remarks: TW = tap water, FB = fine bubble water, Intact = intact seeds, NonFib = seeds with removed fiber plugs

**Fig. 2.** Physiological parameters of the four treatment units. Blue line graphs are showing daily germination percentages, orange lines are displaying total germination percentages, green dash lines are exposing the vigor index, and red arrows indicate the germination peaks of the treatments.
Fig. 3. Germination percentage following soaking duration: a) 1 day; b) 2 days; and c) 3 days. Red lines are indicating the day when 90% of germinated seeds are collected.

Remarks: TW = tap water, FB = fine bubble water, Intact = intact seeds, NonFib = seeds with fiber plugs removed
However, the reason for the incident was not clear. Ahmed et al. (2018) stated that in concordance with a higher germination percentage, fine bubble water’s capability to increase germination is related to the size of the bubbles, which are small enough to pass through seed testa. At the same time, Chanchula, Pavasupree, Pivsa-Art, Wattanawikram, & Porjai (2019) noted that the occurrence is related to the presence of reactive oxygen species (ROS). Siregar, Ramdhani, Rustam, & Sudrajat (2021) mentioned that ROS affects seed membrane cell permeability, allowing the first step of the germination process, the water imbibition. Furthermore, Gomes & Garcia (2013) noted that ROS is beneficial to seeds to protect the materials from pathogens, mobilize reserves in the seed to the embryo, and weaken endosperm for germination. However, it is believed that different seed species may require different ROS concentrations. IIjima et al. (2022) noticed that certain species benefited from fine bubble concentrations. On the other hand, providing different concentrations may harm the species’ growth.

**Effect of Soaking Duration**

In the dry heat method to break oil palm seed dormancy, seeds are soaked in water two times (Corley & Tinker, 2015), seven days and three days of soaking, respectively, with 60 days of heat treatment in between the soakings. In this research, the first soaking and heat treatment was conformable to the procedure, while the second soaking was carried out for 1, 2, or 3 days as treatment levels. The result showed that 3 days of seed soaking (DSS) produced relatively faster first germinated seed selection (17 days), although with a smaller amount of germination (0.1%) compared to those on 2 DSS (18 days, 0.4%) and 1 DSS (24 days, 9.7%) (Fig. 3). It was also found that the time when 90% of final germination occurred was shorter for 3 DSS (38 days) than those on 2 DSS (39 days) and 1 DSS (45 days). Albeit the two occurrences, statistically, the second seed soaking duration does not affect the final germination percentage (Table 1). The insignificant difference in final germination between the three levels of soaking duration treatment is due to the moisture contents of the treated seeds were neither significant. However, 3 DSS shows a higher seed moisture content (SMC) before the seeds were placed in the germination chamber (Fig. 4). In addition, final germinations of the three levels of soaking treatment are considered low, with only 62 to 65% germination (Table 1). The phenomenon is caused by the low SMC of all the treatment levels. It is well recognized that SMC affects germination percentage (Razeek, Sittampalam, & Kapilan, 2016), and moisture content of oil palm seeds during the germination process should be approximately 21-23% (Corley & Tinker, 2015) which parameter was not gained by the 3 levels of the seed soaking treatment (Fig. 4).

Slow imbibition, as shown on levels 1 DSS and 2 DSS, is believed to be normal since oil palm seeds are surrounded by thick shells (Corley & Tinker, 2015). More than 86% of its carbohydrate structure consists of lignocellulose (Ikumapayi & Akinlabi, 2018; Okoroigwe, Saffron, & Kamdem, 2014), a hydrophobic material (Gordobil, Herrera, Llano-Ponte, & Labidi, 2017).

**Fatty acid content**

Fatty acid composition in the tested oil palm kernel exposed 11 types of fatty acids (Table 2), where 8 of them are saturated fatty acids (SFAs) with total 78.0%, two fatty acids are monounsaturated fatty acids (MUFAs) with total 19.1%, while the other is a polyunsaturated fatty acid (PUFA) with 2.9%. Conditions where SFAs is more dominant than unsaturated fatty acid (UFAs) and MUFAs content are higher than PUFA, align with findings in *Garcinia dhanikhariensis* (Bohra, Waman, & Devi, 2021).

The proportion of four main fatty acids in the tested oil palm kernel is shown in Fig. 5. With more than 43% in composition, lauric was the main fatty acid, followed by oleic and myristic acid, with approximately 19% and 16%, respectively. The fourth main fatty acid was palmitic at around 9%. The sequence is aligned with the outcomes of Rahman, Sitompul, & Tjokrodiningrat (2022), which generated the composition percentage of each main fatty acid for oil palm kernel.

**Table 1.** Descriptive statistics of final germination based on the duration of second seed soaking

<table>
<thead>
<tr>
<th>Level</th>
<th>Final germination</th>
<th>N</th>
<th>SD</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 DSS</td>
<td></td>
<td>20</td>
<td>22.38</td>
<td>62.95</td>
</tr>
<tr>
<td>2 DSS</td>
<td></td>
<td>20</td>
<td>20.64</td>
<td>62.97</td>
</tr>
<tr>
<td>3 DSS</td>
<td></td>
<td>20</td>
<td>17.55</td>
<td>64.67</td>
</tr>
</tbody>
</table>

Remarks: DSS = days of seed soaking; N = Number of experimental units; SD = Standard deviation; X = Average of final germination
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Table 2. Composition of fatty acids in oil palm kernel

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Type of Fatty Acid</th>
<th>C:D</th>
<th>Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caproic acid</td>
<td>C6:0</td>
<td>SFA</td>
<td>0.10</td>
</tr>
<tr>
<td>Caprylic acid</td>
<td>C8:0</td>
<td>SFA</td>
<td>2.22</td>
</tr>
<tr>
<td>Capric acid</td>
<td>C10:0</td>
<td>SFA</td>
<td>2.57</td>
</tr>
<tr>
<td>Lauric acid</td>
<td>C12:0</td>
<td>SFA</td>
<td>45.91</td>
</tr>
<tr>
<td>Myristic acid</td>
<td>C14:0</td>
<td>SFA</td>
<td>15.54</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>C16:0</td>
<td>SFA</td>
<td>9.32</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>C18:0</td>
<td>SFA</td>
<td>2.23</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>C18:1</td>
<td>MUFA</td>
<td>19.02</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>C18:2</td>
<td>PUFA</td>
<td>2.88</td>
</tr>
<tr>
<td>Arachidic acid</td>
<td>C20:0</td>
<td>SFA</td>
<td>0.10</td>
</tr>
<tr>
<td>Eicosenoic acid</td>
<td>C20:1</td>
<td>MUFA</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Remarks: SFA = Saturated fatty acid, MUFA = Mono unsaturated fatty acid, PUFA = Poly unsaturated fatty acid
Fig. 5 also shows that even though tap water with seeds in which fiber plugs were removed generated a lower lauric acid proportion (43.36%) and a relatively higher proportion of palmitate and oleic acid, 10.97% and 20.39%, respectively, compared to those on other treatments, the significant difference on observed fatty acid components, i.e., lauric, myristic, palmitate, and oleic, was not found. Furthermore, by utilizing seeds of four vegetable species as experimental objects, Kaymak (2014) concluded that there were correlations between amounts of fatty acids with seed germination percentage, which correlation was not found in oil palm seeds. The correlation coefficient between final germination and four main fatty acids was between -0.05 and 0.18 (Table 3) and was categorized as very low (Anwar, 2009).

**CONCLUSION**

Utilization of fine bubble water, which was generated by an NG3 bubble generator, on the second soaking of oil palm seed in the dry heat method was able to improve seed physiological parameters, where fine bubble treatments generated higher germination percentage on day 22, i.e. 4.67% and 4.40% for intact and fiber plug removed seeds, respectively, compared to those without fine bubble treatments, i.e. 1.87% and 0.33%. For the utilization of fine bubbles on the dry heat method, fiber plug removal during the dormancy breaking process was not necessary. Three days of seed soaking using FB water or tap water showed faster seed germination, which was indicated by early germination, compared to 1 or 2 days of seed soaking.

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