

Shortening Dormancy Period of Oil Palm Seeds (*Elaeis guineensis*) using Sulfuric Acid (H₂SO₄) and Gibberellic Acid (GA₃) Solutions

*Usaha Memperpendek Masa Dormansi Benih Kelapa Sawit (*Elaeis guineensis*) Dengan Menggunakan Larutan Asam Sulfat (H₂SO₄) dan Asam Gibberellat (GA₃)*

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ABSTRAK

Perendaman benih kelapa sawit dalam larutan asam sulfat dan asam gibberellat adalah salah satu cara usaha untuk memperpendek masa dormansi benih kelapa sawit. Tujuan dari penelitian ini adalah menentukan lama perendaman benih kelapa sawit ke dalam larutan asam sulfat yang optimum untuk mempercepat perkecambahannya, menentukan konsentrasi asam gibberellat yang optimum bagi perkecambahan benih kelapa sawit dan menganalisis interaksi penggunaan asam sulfat dan asam gibberellat dalam mempercepat perkecambahan benih kelapa sawit serta mencari kombinasi perlakuan yang terbaik pada semua perlakuan yang dicoba. Rancangan percobaan yang digunakan adalah Rancangan Acak Lengkap (RAL), dengan kombinasi berbentuk faktorial lengkap dengan jumlah perlakuan 16 macam. Setiap perlakuan diulang 4 kali, sehingga terdapat 64 unit percobaan dan setiap unit ditanam 5 benih. Hasil analisis statistik menunjukkan bahwa perendaman benih kelapa sawit dalam larutan asam sulfat dan asam gibberellat berpengaruh terhadap perkecambahan benih tersebut. Lama perendaman benih kelapa sawit selama 48 jam dalam larutan asam sulfat merupakan waktu yang optimum bagi peningkatan kecepatan perkecambahannya dan pemberian asam gibberellat pada konsentrasi 300 ppm masih mempercepat perkecambahan benih kelapa sawit serta tidak terdapat interaksi antara lama perendaman benih dalam larutan asam sulfat dan konsentrasi asam gibberellat. Perendaman benih dalam larutan asam sulfat selama 48 jam dan gibberellat 300 ppm, merupakan kombinasi perlakuan yang terbaik dalam mempercepat perkecambahan benih kelapa sawit.

Kata kunci: perkecambahan, gibberellat, skarifikasi benih, zat pengatur tumbuh

ABSTRACT

Soaking oil palm seeds in a solution of sulfuric acid and gibberellic acid is one way to shorten the dormancy period of oil palm seeds. This study aimed to find out the optimum soaking time of oil palm seeds in sulfuric acid solution to accelerate germination, the optimum concentration of gibberellic acid for oil palm seed germination, the interaction between using sulfuric acid and gibberellic acid in accelerating the germination of oil palm seeds and the best treatment combination in all the tried treatments. The experimental design used was a completely randomized design (CRD), with a complete factorial

combination with a total of 16 treatments. Each treatment was repeated 4 times, so there were 64 experimental units and each unit was planted with 5 seeds. The results of statistical analysis showed that soaking oil palm seeds in a solution of sulfuric acid and gibberellic acid had an effect on the germination of these seeds. The duration of soaking oil palm seeds for 48 hours in sulfuric acid solution constituted the optimum time for increasing the speed of germination and giving gibberellic acid at a concentration of 300 ppm still accelerated the germination of oil palm seeds and there was no interaction between the length of soaking the seeds in sulfuric acid solution and the concentration of gibberellic acid. Soaking the seeds in a solution of sulfuric acid for 48 hours and gibberellate 300 ppm was the best combination treatment to accelerate the germination of oil palm seeds.

Keywords: germination, gibberellate, seed scarification, growth regulator substance

INTRODUCTION

Oil palm (*Elaeis guineensis*) in Indonesia is one of the leading plantation commodities which is a source of foreign exchange (Utami, Kumala Putri and Ekayani, 2017) and can also create jobs and increase welfare for smallholder farmers (Darlita, Joy & Sudirja, 2017). The development of oil palm plantations in Indonesia from year to year continues to increase. In 2018, based on the data of the Indonesian Central Statistics Agency, the total area of oil palm plantations reached 41,667,011 ha from 31,730,961 ha in 2016 (Turnip & Arico, 2019). The rapid development of oil palm plantations showed a revolution in oil palm plantations in Indonesia (Purba & Sipayung, 2017). In supporting the program to develop oil palm plantations, one aspect that needs special attention is the provision of healthy seeds with superior potential and on time (Rosa & Zaman, 2017). Propagation of oil palm seedlings is generally still done generatively because the tissue culture propagation method is still very expensive (Hadi, Widajati and Selly Salma, 2017). Oil palm seeds have hard skin and are dormant so that the germination process of oil palm seeds is quite difficult (Farhana, Ilyas and Budiman, 2013). The dormant nature is due to the presence of a barrier in the form of a germ pore structure, namely the operculum covering the embryo as a result, the radicle and plumule have difficulty getting out of the seed shell (Agustiansyah, 2020).

Breaking the dormancy of oil palm seeds can be done physically, through mechanical scarification by sanding, filing, cutting and puncturing certain parts of the seeds and chemical scarification is usually done using hot water and chemicals such as strong acids, alcohol and H₂O₂ which aims to damage or soften the seed coat (Kartika, M and M, 2015). In addition, growth regulators (ZPT) can also be used in the breaking seed dormancy in which these growth regulators can accelerate germination and increase the percentage of seed germination, as a result, the plant growth rate will also increase (Asra, 2014). One of the growth regulators that can increase the seed germination is gibberellic acid (GA₃) (Elfianis, 2019).

Sulfuric acid (H₂SO₄) is one of the strong acids that can be used to break dormancy of hard-skinned seeds (Hamzah, 2014). Oil palm seeds have hard skin because the seed coat is composed of cellulose, which binds firmly to lignin and silica impermeable to water and gas. The cellulose can be hydrolyzed by sulfuric acid into monomers in the form of monosaccharides in the form of glucose (Lisneri, 2016). This condition makes the seed coat soft and permeable to water and gas, which is the initial process for seed germination (Fajrina and Soetopo, 2018). Gibberellic acid in seed germination is useful for stimulating the synthesis of ribonuclease, amylase and protease enzymes in the endosperm of the seeds to be used in the germination process. The

addition of gibberellic acid to seeds is expected to accelerate the process of germination metabolism (Sari, Hanum & Charloq, 2014). This study aimed to find out the duration of soaking oil palm seeds in the optimum sulfuric acid solution to accelerate germination, the optimum concentration of gibberellic acid for oil palm seed germination and to assess whether there was an interaction between the use of sulfuric acid and gibberellic acid in accelerating the germination of oil palm seeds and search the best treatment combination in all the treatments tried.

MATERIALS AND METHODS

This study was conducted at the Laboratory of Agronomy, Faculty of Agriculture, Jenderal Soedirman University, Purwokerto, Java, Indonesia. The average laboratory room temperature was 26⁰C with an average humidity of 52.5%. The oil palm seeds used were the 320 seeds of Tenera variety with the same maturity and the same size. The seeds were peeled from the skin and fibers until they were clean. Then the seeds were dried in the sun for 7 days before being treated. The experimental design used Completely Randomized Design, a complete factorial combination with 16 kinds of treatment. Each treatment was repeated 4 times so that there were 64 experimental units and each unit was planted with 5 seeds. For the purpose of observing, 1 seed sample was taken by random sampling from each experimental unit. The details of the tried treatment were a combination of two factors, namely 1). The duration of soaking the seeds in sulfuric acid solution consisting of 4 levels, T₀ = not soaked (as a control), T₁ = soaked for 24 hours, T₂ = soaked for 48 hours and T₃ = soaked for 72 hours. 2). Immersion in gibberellic acid for 24 hours, at several concentrations, namely G₀ = immersion in water/distilled water (as a control), G₁ = 200 ppm, G₂ = 250 ppm and G₃ = 300 ppm. Consequently, there were 16 kinds of treatment combinations, namely

T₀G₀, T₀G₁, T₀G₂, T₀G₃, T₁G₀, T₁G₁, T₁G₂, T₁G₃, T₂G₀, T₂G₁, T₂G₂, T₂G₃, T₃G₀, T₃G₁, T₃G₂ and T₃G₃. The germination site was in the form of a plastic pot with a diameter of 25 cm and a height of 20 cm filled with a planting medium of a mixture of soil and sand in the ratio 1:1 to $\frac{3}{4}$ height of the surface of the plastic pot.

Preparation of 13.6169 N sulfuric acid solution

The preparation of 1 liter of 13.6169 N sulfuric acid solution was carried out by diluting 36 N concentrated sulfuric acid with the formula $V_1.N_1 = V_2.N_2$, where N₁ = 36 N, V₂ = 1 liter and N₂ = 13.6169 N, then the sulfuric acid to be diluted (V₁) was as much as 377.8 ml. Then the 377.8 ml of 36 N sulfuric acid was carefully inserted into a 1-liter volumetric flask through the wall of the tube filled with 500 ml of distilled water. The remaining sulfuric acid in the measuring cup was rinsed with a little distilled water. Then it was put in a volumetric flask of the same size and added distilled water until the volume reached 1 liter. The volumetric flask was covered and shaken until homogeneous.

Preparation of gibberellic acid solution

The gibberellic acid was weighed with an electric scales of 200 mg, 250 mg and 300 mg (Pertiwi, Tahir and Same, 2016). The gibberellic acid solution was dissolved in alcohol first. It was put into a 1-liter volumetric flask already filled with 500 ml of distilled water. The volumetric flask and stirring rod were rinsed with distilled water up to 3 times so that there was no more gibberellic acid left. The solution was added with distilled water again until the volume was exactly 1 liter, then the it was shaken to make it homogeneous.

Giving treatment

The seeds were immersed in sulfuric acid solution according to the predetermined treatments (24 hours, 48 hours and 72 hours). After the soaking, the

seeds were washed under the running water. Then they were immersed in a gibberellic acid solution for 24 hours adjusted to the existing concentration treatment (200 ppm, 250 ppm and 300 ppm).

Seeding seeds

After the seeds were treated, they were planted on the available media with the seeds facing down (horizontally). The planting was carried out by pressing the seeds with fingers until $\frac{3}{4}$ of the seeds enter the media. The $\frac{1}{4}$ part of the seed was still visible. To maintain the moisture, watering was conducted once a day (morning or evening).

Observation variable

The observations were made 1 week from the time the seeds were sown for 8 months. The observed variables were 1) The speed of the seeds germinating as indicated by the vigor index. The observations were made after 1 month of seed sowing at intervals of 7 days, for 7 months or until the control treatment grew. 2) The percentage of seeds that germinated, was the number of seeds that germinated until the end of the observation. 3) After the seeds germinated, the length of the plumules was measured. The measurements were made from the base of the plumule to the tip. 4) At the end of the observation the seeds were removed and the height was measured from the base of the stem to the tip of the plant (the point of growth). 5) The length of the roots was measured from the root neck to the longest root after completing the observation. 6) The wet weight of the canopy and the wet weight of the roots, the roots were separated from the canopy, then each part was weighed. 7) the shoot dry weight and root dry weight, the root and shoot dry weight were oven-baked at 75°C for 72 hours, then each part of the dry weight was weighed.

Data Analysis

The data from the observations were tested with the F test, if they were

significantly different, it was then proceeded with the DMRT test at the 5% level and continued with a regression test.

RESULTS

Soaking oil palm seeds in a solution of sulfuric acid and gibberellic acid affected the germination of these seeds. Both of these solutions affected all the observed variables (Table 1).

Effect of Soaking Seeds in Sulfuric Acid Solution

The effect of soaking time for oil palm seeds in sulfuric acid solution on the speed of the seeds to germinate was very significant. The relationship between the immersion time and the germination rate formed a quadratic function Figure 1 with the equation $Y = 0.010894 + 0.000756 X - 7.93.10^{-06} X^2$. The optimum point was achieved when the seeds were soaked for 47.68186 hours or approximately 48 hours with a germination rate of 0.0289, while the unsoaked seeds had a speed of 0.01. The germination speed gradually began to fall after the soaking was carried out for more than 47.68186 hours.

Soaking the seeds for 48 hours based on the observations was able to accelerate germination 15 weeks earlier than the seeds that were not soaked or germinated after 1 month of seeding. The speed of this germination would affect other observed variables. The duration of soaking oil palm seeds affected the percentage of germination. The magnitude of the effect of the duration of soaking the seeds in sulfuric acid solution is shown in Figure 2. The percentage of seeds that were not soaked was very low. This relationship formed a quadratic function with the equation $Y = 19.41667 + 0.600694 X - 0.00651 X^2$. The optimum point was reached at (46.13; 33.27).

Figure 3 show the relationship between the length of immersion of oil palm seeds in sulfuric acid solution to the length of the plumules. The length of the plumules from

germination of soaked seeds was significantly different from the length of the plumules that were not soaked (control), but between the immersion treatments (24, 48 and 72 hours) they were not significantly different. The longest plumule reached 1.369183 cm \approx 1.4 cm in immersion for 47.76 hours \approx 48 hours. The curve of the relationship between the length of immersion of oil palm seeds and the seed height formed a quadratic function (Figure 4) with the equation $Y = 5.994167 + 0.233958 X - 0.002546 X^2$. The highest seedling height reached 11.3683 cm \approx 11.4 cm in immersion for 45.94091 hours \approx 46 hours.

The effect of soaking time for oil palm seeds on root length was very significant (Figure 5). The effect was to form a quadratic curve with the equation $Y = 14.00083 + 0.552118 X - 0.006735 X^2$, the optimum point was reached at point (40.99076; 25.3167).

The results of the regression test on the wet and dry weight of the canopy (Figure 6 and 7), showed a quadratic curve with the respective equations $Y = 2.24875 + 0.212969 X - 0.001921 X^2$ and $Y = 0.757083 + 0.038142 X - 0.000423 X^2$.

The optimum point of canopy wet weight (Figure 6) was reached at point (55.444407; 8.152677), while the dry weight (Figure 7) was reached at point (45.06667; 1.616558). Likewise, the relationship between the length of immersion of oil palm seeds with wet weight and dry weight of the roots (Figure 8 and 9) formed a quadratic curve with each equation obtained was $Y = 0.682083 + 0.032587 X - 0.000365 X^2$ and $Y = 0.194167 + 0, 011389 X - 0.000145 X^2$. The optimum point was reached at the point (44.60198; 1.408801) for the wet weight of the roots (Figure 8), while the optimum dry weight of the roots (Figure 9) was 0.4183 grams for 39.36 hours of immersion.

Table 1. The results of the statistical analysis of soaking oil palm seeds in a solution of sulfuric acid and gibberellic acid on the observed variables

Observed Variables	Treatment		
	T	G	T x G
The speed at which the seeds germinated	**	*	ns
Percentage of seeds germinating	**	**	ns
Plumula length	**	*	ns
Seed height	**	*	ns
Root length	**	*	ns
Canopy wet weight	**	*	ns
Root wet weight	**	**	ns
Canopy dry weight	**	*	ns
Root dry weight	*	*	ns

Remarks: T = immersion in sulfuric acid solution (in paragraph form), G = immersion in gibberellic acid solution, TxG = interaction between T and G, ** = very significant difference * = significantly different and ns = not significant

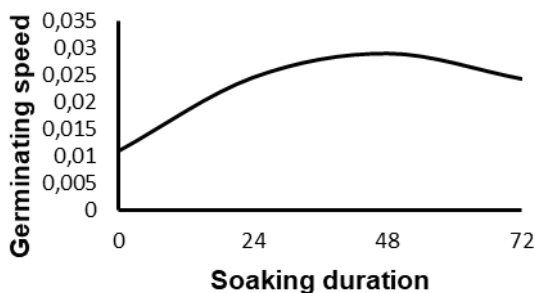


Figure 1. The graph of the relationship of soaking time in sulfuric acid solution towards the speed of germinating seeds

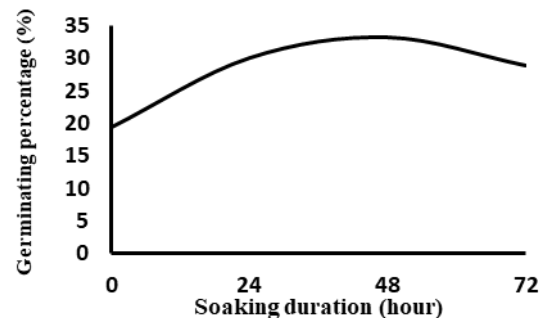


Figure 2. The graph of the relationship between the length of time soaking the seeds in sulfuric acid solution to the percentage of seeds

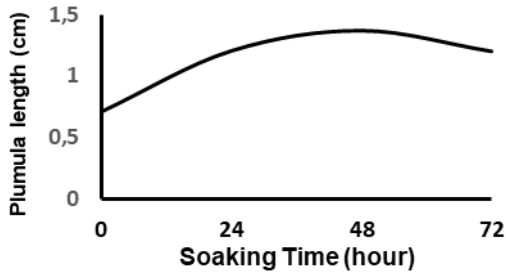


Figure 3. The graph of the relationship between the length of soaking the seeds in sulfuric acid solution and the length of the plumules

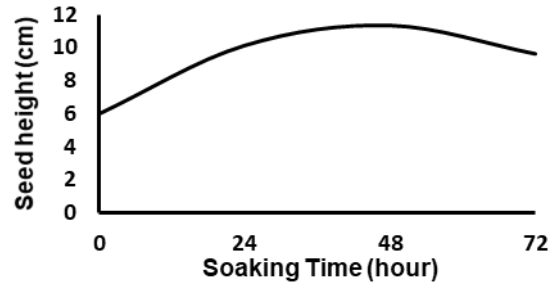


Figure 4. The graph of the relationship between the soaking time of the seeds in sulfuric acid solution and the height of the seeds

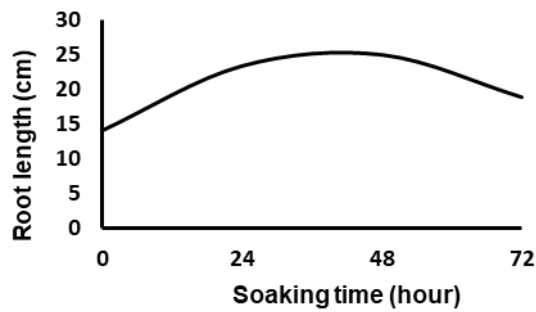


Figure 5. The graph of the relationship between seeds soaking time in sulfuric acid solution and the root length

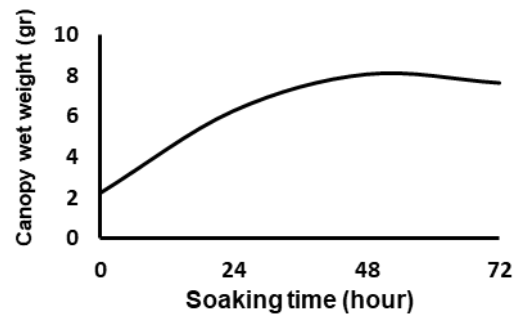


Figure 6. The graph of the relationship between the soaking time of seeds in sulfuric acid solution and canopy wet weight

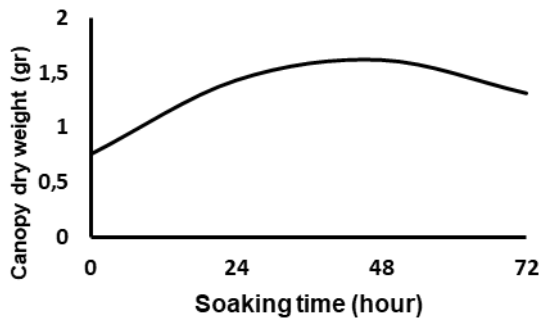


Figure 7. The graph of the relationship between the soaking time of seeds in sulfuric acid solution and canopy dry weight

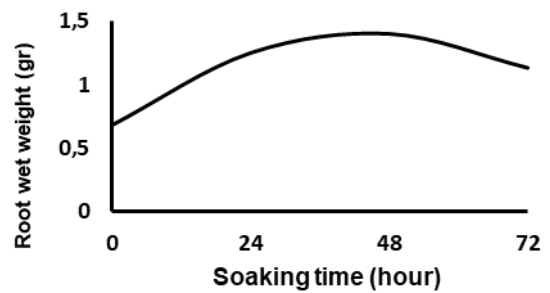


Figure 8. The graph of the relationship between the soaking time of seeds in sulfuric acid solution and root wet weight

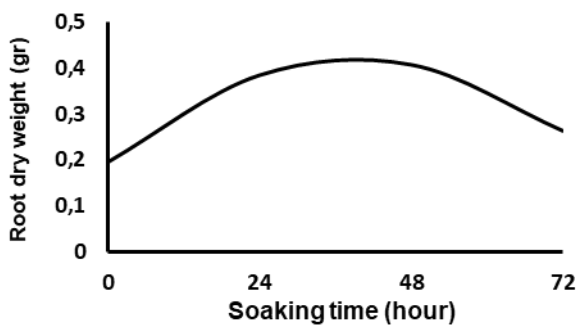


Figure 9. The graph of relationship between the soaking time of seeds in sulfuric acid solution and root dry weight

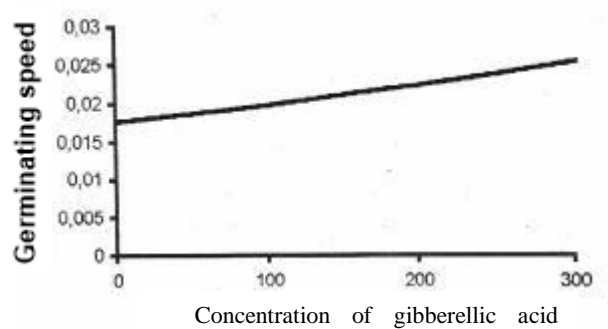


Figure 10. The graph of relationship between the concentration of gibberellic acid and the germinating speed of oil palm seeds

Effect of Soaking Seeds in a Gibberellic Acid Solution

The effect of immersing oil palm seeds in a solution of gibberellic acid on germination was significant to very significant. This could be seen in all observed variables, starting from the speed of the seeds germinating (Figure 10), the percentage of seeds germinating (Figure 11), the length of the plumules (Figure 12), the height of the seeds (Figure 13), the length of the roots (Figure 14), the wet and dry weight of the shoots (Figure 15 and

Figure 16) and the wet weight and the dry weight of the roots (Figure 17 and Figure 18). The effects of gibberellic acid on all these variables were in the form of a linear function, where the higher the concentration of gibberellic acid was given (Figure 11 to 18), the higher the effect would be. In other words, the optimum concentration of gibberellic acid was not achieved. The effects of gibberellic acid concentration on various observed variables were in the graphs below (Figure 11 to 18).

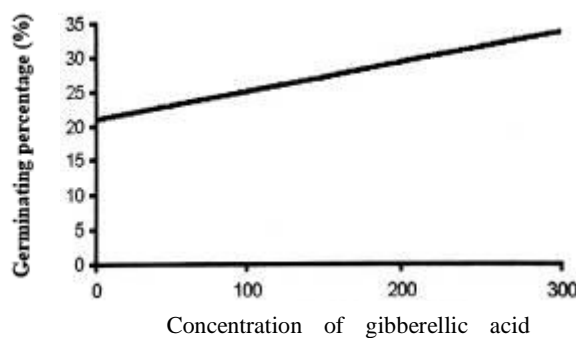


Figure 11. The graph of relationship between the concentration of gibberellic acid and the percentage of germinating seeds

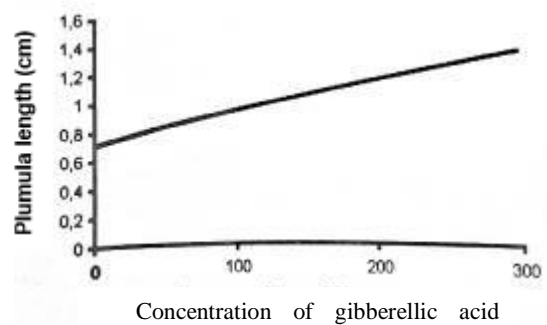


Figure 12. The graph of relationship between the concentration of gibberellic acid and plumula length

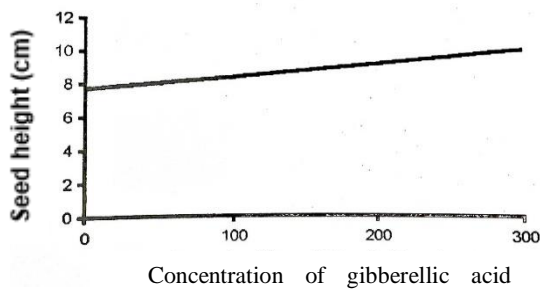


Figure 13. The graph of relationship between the concentration of gibberellic acid and seed height

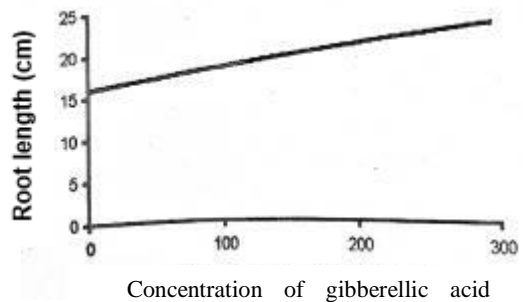


Figure 14. The graph of relationship between the concentration of gibberellic acid and root length

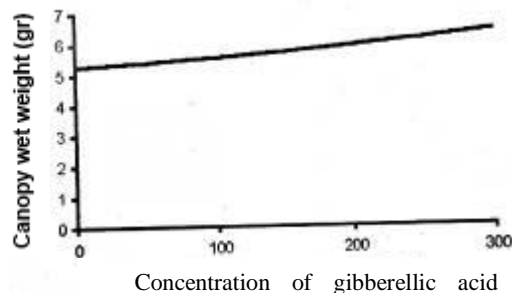


Figure 15. The graph of relationship between the concentration of gibberellic acid and canopy wet weight

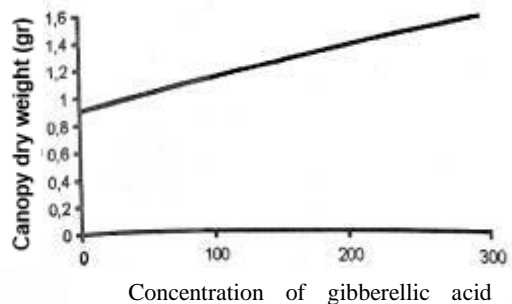


Figure 16. The graph of relationship between the concentration of gibberellic acid and canopy dry weight

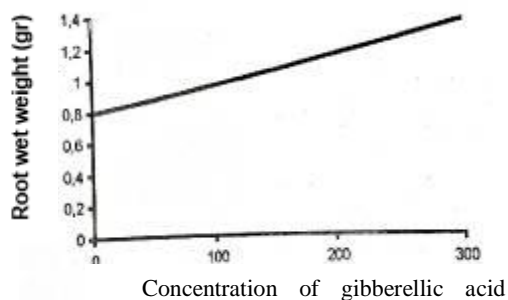


Figure 17. The graph of relationship between the concentration of gibberellic acid and root wet weight

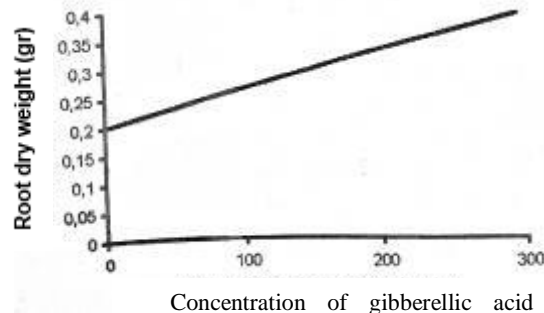


Figure 18. The graph of relationship between the concentration of gibberellic acid and root dry weight

DISCUSSION

The soaking oil palm seeds in sulfuric acid solution had an effect on the speed of the seeds to germinate, the percentage of seed germination, the length of the plumules, the height of the seeds, the length of the roots, the wet and dry weight of the shoots and the wet weight and dry weight of the roots with the optimum soaking time for 48 hours.

The use of sulfuric acid (H_2SO_4) functions to break cellulose or polysaccharide bonds that bind lignin and silicates strongly (Figure 19) (Hedty, Mukarlina & Turnip, 2014) found in oil palm seed coat, so that the seed coat becomes more permeable to water and gas. The permeability of the seed coat to water and gas causes active digestive enzymes and loss of inhibiting substances (Agroekoteknologi, Usu and No, 2017). In addition, it also causes the work of the hormone (GA3) in the seeds to be more effective (Gulston, 2017).

The speed of the seeds germinating (seen from the graph) continued to increase until they were immersed for 47.68186 hours \approx 48 hours. After this time the germination rate decreased. The decrease in speed was presumed resulting from a small portion of the sulfuric acid solution entering the seeds due to the long soaking that was conducted. The entry of sulfuric acid into the seeds

could damage the seed embryo cells. This causes the germination of seeds to decrease (Gulston, 2017).

Soaking the seeds in sulfuric acid also had an effect on increasing the percentage of germination (Figure 2). Although the soaking had an effect on increasing the percentage of germination, the percentage of seed germination produced was still small (below 50%). It was assumed to have occurred due to poor seed quality, including the low seed viability (Dethan, R.L.Solle and Hendrik, 2020). The oil palm seed setbacks could occur from the beginning of the procurement process until the time they were germinated. In addition, other factors that could affect the viability of seeds were the conditions of pollination and also the nature of the seed genotypes, which each individual varies widely (Julyan, Qadir and Supijatno, 2017).

The increased values of other variables, namely plumule length, seedling height, root length, canopy wet and dry weight and wet and dry weight of roots, were due to soaking the seeds in sulfuric acid functioning as a scarification solution to increase the permeability of the seed coat to water and gas and the disappearance of germination inhibiting factors in the seeds for the seeds to germinate faster. This speed of germination will affect further seedling growth.

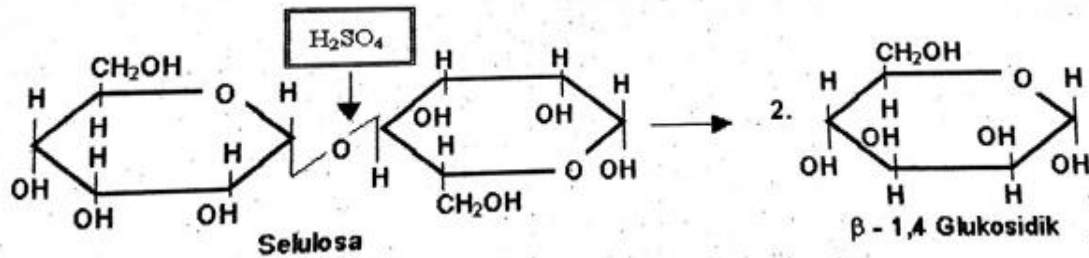


Figure 19. The process of hydrolysis of cellulose by H₂SO₄ into glucose

The germination of oil palm seeds treated with gibberellic acid showed a significantly higher number of observational variables compared to the control (0 ppm). This fact indicates that gibberellic acid has the ability to encourage or induce oil palm seed germination. This reason was in accordance with the opinion of some researchers, such as Heddy (1996:25) stating that gibberellic acid was involved in activating the synthesis of hydrolytic enzymes which function to produce soluble substances, thus supporting embryonic development and the emergence of sprouts (Polhaupessy & Sinay, 2014).

The effects of gibberellic acid on other observed variables, such as seed height, root length, wet weight and dry weight of the roots and wet weight and dry weight of the roots were indirect or due to the opportunity for seeds to grow and develop earlier by having the seeds applied in gibberellic acid that only drove up the rate of seed germination. Presumably, if gibberellic acid was given to growing oil palm seedlings, it will affect the growth of oil palm seeds. This was because the function of gibberellic acid was only to trigger the start of a growth process, while the subsequent growth process depends on other factors, such as the availability of nutrients, water and other environmental conditions. The duration of soaking the seeds in sulfuric acid solution and the concentration of gibberellic acid based on the results of statistical analysis (Table 1) did not show any significant interactions with all the observed variables. Presumably, it was due to washing the seeds after soaking in sulfuric acid solution which was not long enough that relatively large

amounts of sulfuric acid remained in the seed coat. Thus, the gibberellic acid that enters the seed coat was damaged. Consequently, sulfuric acid was a very strong dehydrator for organic compounds, but the result of the 5% DMRT test indicated that the best treatment combination was T₂G₃, namely soaking the seeds in sulfuric acid solution for 48 hours followed by soaking them in gibberellic acid at a concentration of 300 ppm.

CONCLUSION

Soaking oil palm seeds for 48 hours in a solution of 13.6 N sulfuric acid is the optimal time to increase the rate of germination. Gibberellic acid treatment at a concentration of 300 ppm still accelerated the germination of oil palm seeds. Then there was no interaction between the length of soaking the seeds in a sulfuric acid solution and the concentration of gibberellic acid. Soaking the seeds in a sulfuric acid solution for 48 hours and a gibberellic acid concentration of 300 ppm were the best combination treatment in accelerating the germination of oil palm seeds.

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