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Physiological responses of bio-silica-treated oil palm seedlings to drought stress

Tanggap fisiologi bibit kelapa sawit yang diberi bio-silika terhadap cekaman kekeringan

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Abstract

Silica (Si) in the form of soluble silicic acid [H_4SiO_4] was an element that makes plants more resistant to drought stress through biochemical or molecular processes and contributing to growth stimulation under biotic and abiotic stress conditions. The objective of this study was to determine the response of oil palm seedlings to drought stress by the bio-Si application. The experiment was arranged in complete random design (CRD) with ten replicates. Bio-Si was developed in solid and liquid forms with a dissolved Si content at least 10% (w/v). The eight combinations of solid bio-Si application per seedling were: (i) blank (without fertilizers), (ii) 5 g NPK 15-15-15, (iii) 5 g NPK 15-15-15 + 10^9 cfu of Si-solubilizing microbes (SSM), (iv-viii) 5 g NPK 15-15-15 + 2.5; 5.0; 7.5; 10 g bio-Si; and 5 g NPK 15-15-15 + 5 g Na_2SiO_3. Meanwhile, liquid bio-Si application per seedling were: (i) blank (without fertilizers), (ii) 5 g NPK 15-15-15, (iii) 5 g NPK 15-15-15 + 10^9 cfu of SSM, (iv-viii) 5 g NPK 15-15-15 + 25 mL; 50 mL; 75 mL; 100 mL bio-Si; and 5 g NPK 15-15-15 + 50 mL Na_2SiO_3. Drought stress tolerance was analyzed by using proline concentration, nitrate reductase activity (NRA), chlorophyll content, and stomatal closure in the leave of oil palm seedlings. Based on the physiological response, this research indicates that bio-Si application could induce seedling tolerance to drought stress. The bio-Si treatments gave a positive response of proline concentration, nitrate reductase activity (NRA), chlorophyll content, and stomatal closure. The doses of 5 g NPK 15-15-15 + 7.5 g solid bio-Si and 5 g NPK 15-15-15 + 75 mL liquid bio-Si per seedling were a recommended to increase oil palm seedlings tolerance to drought stress.

[Key words: bio-Si, chlorophyll, nitrate reductase activity, Si-solubilizing microbes]

Abstrak

Silika (Si) dalam bentuk asam silikat [H_4SiO_4] *merupakan unsur yang dapat menyebabkan tanaman lebih tahan terhadap cekaman kekeringan melalui proses biokimia atau molekuler dan menstimulasi pertumbuhan dalam kondisi cekaman biotik dan abiotik. Tujuan dari penelitian ini adalah mengetahui respons fisiologi bibit kelapa sawit yang diberi bio-Si terhadap cekaman kekeringan. Penelitian didesain dengan rancangan acak lengkap (RAL) dan sepuluh ulangan. Bio-Si dikembangkan dalam bentuk padat dan cair dengan kadar Si terlarut minimal 10 % (b/v). Delapan aplikasi bio-Si padat per bibit adalah: (i) blanko (tanpa pupuk), (ii) 5 g NPK 15-15-15, (iii) 5 g NPK 15-15-15 + 10^9 cfu mikrob pelarut silika, (iv-viii) 5 g NPK 15-15-15 + 2,5 g; 5,0 g; 7,5 g; 10 g bio-Si, dan 5 g NPK 15-15-15 + 5 g Na_2SiO_3. Sementara untuk aplikasi bio-Si cair per bibit adalah: (i) blanko (tanpa pupuk), (ii) 5 g NPK 15-15-15, (iii) 5 g NPK 15-15-15 + 10^9 cfu mikrob pelarut silika (MPS), (iv-viii) 5 g NPK 15-15-15 + 25 ml; 50 ml; 75 ml; dan 100 mL bio-Si, dan 5 g NPK 15-15-15 + 50 ml Na_2SiO_3. Pengamatan yang dilakukan meliputi analisis prolin, aktivitas nitrat reduktase (ANR), kandungan klorofil, serta penutupan stomata pada daun bibit kelapa sawit. Berdasarkan data fisiologi yang diperoleh dari kegiatan penelitian ini, aplikasi bio-Si dapat meningkatkan ketahanan bibit kelapa sawit terhadap cekaman kekeringan. Perlakuan bio-Si memberikan respon positif terhadap konsentrasi prolin, aktivitas nitrat reduktase (ANR), kandungan klorofil, serta morfologi stomata. Dosis 5 g NPK 15-15-15 + 7,5 g bio-Si padat dan 5 g NPK 15-15-15 + 75 mL bio-Si cair dapat direkomendasikan untuk meningkatkan ketahanan bibit kelapa sawit terhadap cekaman kekeringan.*

[Kata kunci: bio-Si, klorofil, aktivitas nitrat reduktase, mikrob pelarut silika]

Introduction

Drought stress is commonly attributed to conditions where the available water in the soil was a shortage due to lack of rainfall. In plants, this condition causes growth inhibition, abnormal plant development, and loss of crop productivity (Jaleel *et al*., 2009). However, plants may vary in their tolerance to drought stress depending upon intensity and duration of drought, species and growth phases of a plant (Kadir, 2011). Currently and for some years ahead, it is predicted that drought stress will be enlarging due to global

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warming. Xu *et al*. (2010) reported that the area of land affected by extreme drought will increase from 1 to 30%, due to a high rate of evapotranspiration.

The effect of drought stress in many plants has been reported, including morphological and physiological changes, yield and related traits, as well as pigment composition (Mafakheri *et al.*, 2010; Rahdari & Hoseini, 2012). The morphological changes were characterized by reducing in plant height (Wu *et al*., 2008) and stem length (Sankar *et al*., 2008) due to decreasing on cell division and cell enlargement which impaired enzyme activities, loss of turgor and decreased energy supply (Farooq *et al*., 2009). Furthermore, drought stress decreases the rate of photosynthesis resulting in less assimilate production for growth and yield.

Oil palm (*Elaeis guineensis* Jacq.) with their shallow fibrous roots is known as a sensitive plant to drought conditions (Maryani, 2012) which adversely affects growth and production. In Indonesia, drought period occurs during growth and development of oil palm plants and potentially causes loss of productivity up to 40%. Actually, in oil palm water shortage is very harmful as it delays fruit ripening, reduces fruit bunch weight, decreases oil level, and decreases fruit bunch numbers for up to next nine months. Stress symptoms can also be expressed by delayed flowering if stress occurs before the normal flowering time. In oil palm, water stress may induce an increase in male inflorescence production and hence lower the sex ratio. In oil palm, water stress may increase abortion of the female inflorescences and bunch failure. This is due to limited assimilates as a result of the inhibition of current photosynthesis during stress (Noor *et al*., 2011). However, the drought tolerance in plants may vary each other. By comparing two oil palm hybrids in a drought condition, Silva *et al.* (2017) reported that one hybrid showed extremely decreases in activities of key enzymes associated with carbon metabolism, including Rubisco, ADP-glucose pyrophosphory-lase, and sucrose-phosphate synthase, then another hybrid. More tolerance hybrid plants were better in adapting its physiological, morphological, and biochemical traits to cope with drought.

Responses of plants to drought stress usually related to their adaptation in physiological, biochemical and molecular levels. Seedlings adapt to stress environment by different mechanisms, including changes in morphological and developmental pattern as well as physiological and biochemical processes (Cao *et al.*, 2011). Proline, nitrate reductase activity (NRA), chlorophyll content, and stomata closure are representing an analytical parameter to observe the physiological tolerance of plant in a period of drought stress. Proline is an amino acid found in various types of plant tissue as a response to drought stress and heavy metals (Shyam & Aery, 2012). Free proline content can increase upon exposure of plants to drought, salinity, cold, heavy metals, or certain pathogens. The plant cells also accumulated proline as a compatible solution for achieving osmotic adjustments in stress conditions, which is stimulated by drought, salt, cold and ABA (Bajguz & Hayat, 2009). Meanwhile, nitrate reductase activity (NRA) is directly related to the growth and yield that could be achieved by a plant. The key factors in efficient N utilization are nitrate uptake and allocation (Wang *et al*., 2012). Disruption in N-metabolism is a crucial in-plant injury under drought stress conditions (Lisar *et al*., 2012). NRA is the major enzyme in nitric metabolism providing essential organic nitrogen for plant growth (Shyam & Aery, 2012). NRA increased the assimilation of nitrates in plants, which was affecting by the level of nitrogen compounds such as caffeine, protein, chlorophyll, proline, and others on the leaves (Pandey, 2010).

Closing of stomatal opening becomes a primary and rapidly occurring response to drought stresses aimed at regulating the leaf temperature (via transpiration), the flow of CO_2 and water loss (Zandalinas *et al*., 2018). Stomatal closure is the initial response of plants under water deficit conditions and is followed by changes in root growth, leaf surface area, ultra-chloroplast structure, and protein pigment (Farooq *et al*., 2009; Ai & Banyo, 2011). Usually, plants at the first growth phases are a lower rate of water use then more adapted to drought due to efficient transpiration. Chlorophyll is an essential factor as a light absorbing pigment in the process of photosynthesis (Benny *et al*., 2015). Chlorophyll is one of the pigments in the chloroplast that fill the complex reaction center as an accumulator of solar radiation energy (Putra *et al*., 2015). While the chlorophyll b is the result of biosynthesis of chlorophyll a (Ai & Banyo, 2011).

Application of silicon (Si) in available form is to improve drought tolerance of the plant. Silicon is one of the two most common chemical elements in the earth's crust (lithosphere), around 27.6%, and is absorbed by almost all plants in form of monosilicic acid or H_4SiO_4 (Makarim *et al*., 2007). Silicon plays an important role in improving water equilibrium in plant tissues, photosynthesis activity, cell wall strengthening, and root spread. Silicon can also prevent water loss by reducing cuticle transpiration, increasing CO_2 assimilation rate and the opening of stomata wider. The leaf surface of plants that less protected from Si caused a lot of water loss due to high transpiration. It indicates that the existing of Si makes plants more resistant to drought (Makarim *et al*., 2007).

Available Si product that is readily absorbed by plants is mostly still imported and expensive. Local materials such as quartz minerals contain abundant Si and are available in large quantity in Indonesia.

However, activation with bacteria and fungi is assumed to be more efficient to increase the solubility of Si in quartz minerals, as these microbes produce organic acids that are environmentally friendly. The aim of this study was to determine the physiological response of oil palm seedlings to drought stress after the application of bio-Si derived from quartz mineral which enriched with selected Si-solubilizing microbes.

Material and Methods

Plant materials

The research was conducted at the greenhouse of Indonesian Biotechnology Research Institute for Estate Crops, Bogor, West Java from February - September 2017. Oil palm seedlings of Dura x Pisifera species were obtained from IOPRI (Indonesian Oil Palm Research Institute), Medan, North Sumatra. The oil palm seedlings were five months old taken from the main nursery at the greenhouse. From the pre-nursery stage, the seedlings were planted into polybags (40 x 40 cm) containing 20 kg top soil: sand: compost media (1:1:1). The soil was collected from a Ultisol soil in Ciomas, Bogor, West Java.

Bio-Silica preparation

The silica source was quartz type collected from Bangka-Belitung province. Silica extract was prepared by using method reported by Santi & Goenadi (2017) and Santi *et al*. (2017). Solid form of bio-Si mixed with 5% (w/w) selected Si-solubilizing microbes, i.e. *Aeromonas punctata* (10^8 cfu), *Burkholderia cenocepacia* (10^8 cfu), *Burkholderia vietnamiensis* (10^7 cfu), and *Aspergillus niger* (10^6 propagule). Whereas liquid silica form was prepared by using 60 g silica extract dissolved in 400 mL distilled water. The soluble silica (H_4SiO_4) was determined by spectrophotometer (Anonim, 1992).

Experimental design

The experiment was arranged in complete randomized design (CRD) with ten replicates. The first group of treatments was the combination of solid bio-Si dosage and the second one was the combination of liquid bio-Si dosage with standard NPK fertilizers. Each of the first and second groups consisted of eight treatments. The eight-treatments of solid form of bio-Si were: (S1) blank (without fertilizers), (S2) 5 g NPK 15-15-15, (S3) 5 g NPK 15-15-15 + 10^9 cfu SSM, (S4-S8) 5 g NPK 15-15-15 + 2.5; 5.0; 7.5; 10 g bio-Si, and 5 g Na_2SiO_3. The liquid form of bio-Si treatments were: (L1) blank (without fertilizers), (L2) 5 g NPK 15-15-15; (L3) 5 g NPK 15-15-15 + 10^9 cfu SSM; (L4-L8) 5 g NPK 15-15-15 + 25; 50; 75; 100 mL bio-Si, and 50 ml Na_2SiO_3 respectively.

Application of Bio-Si

Application of bio-Si was carried out before drought stress condition. Every month until five months after transplanting, bio-Si was given with the same dose depending on the treatment in the main nursery. The liquid formula was sprayed to the seedlings and a solid formula was sowed to the soil. Oil palm seedlings were fertilized by using NPK compound fertilizer 15-15-15. The seedlings were also watered regularly every day, 1 to 3 L per polybag from the first until the fifth months. The drought stress condition was given based on a method reported by Putra *et al*. (2015). For the first until four months after transplanting, seedlings were watered regularly every day as much as 1-3 liters per polybag. Drought stress treatment was started at the beginning of the fifth month after transplanting. During the drought stress treatment, no watering was done until the moisture content of the soil at seedling media reached a permanent wilting point. The seedlings being tested were placed in a plastic house to anticipate the possibility of rain during the experiment.

Proline analysis

Proline analysis was developed by Abraham *et al*. (2010). Palm oil leaves were weighed for 0.5-1 g. The leaves were crushed and soaked in 10 mL sulfosalicylic acid 3% solution then homogenized. The homogeneous solution was filtered with Whatman paper No.2. A total of 2 mL filtrate was transferred into a new tube and 2 mL ninhydrin acid solution and 2 mL glacial acetic acid were added. The filtrate was incubated at 100ºC for 1 hour in the water bath. The solution was added with 4 mL toluene and stirred for 15-20 sec. The absorbance of the solution was measured with a wavelength of 520 nm using a spectrophotometer.

Nitrate reductase activity (NRA)

Nitrate reductase activity (NRA) was observed before treatments (fifth months in the nursery) and after treatments (sixth months in the nursery). A total of 200 mg oil palm leaves was thinly sliced and put into a test tube filled with 5 mL solution of 0.1 M phosphate buffer pH 7.5 and incubated in dark condition. After 24 h the solution was replaced with a new 5 mL 0.1 M phosphate buffer pH 7.5 and added with 0.1 mL 0.05 M $NaNO_3$ solution. The solutions were incubated for 2 hours in the dark at room temperature. Subsequently, 0.1 mL solution was transferred into a test tube which had previously been filled with dye solution (0.2 mL sulfinyl amide 1%- and 0.2 mL N-naphthyl ethylene diamide 0.02%). The color of the solution turned pink after 10-15 minutes and then 2.5 mL distilled water was added until reaches 3 mL of volume. Observations of NRA contents were carried out with a spectrophotometer at

wavelength of 540 nm (Miranda *et al.,* 2001). The NRA was calculated by the equation (cite the reference):

$$\text{NRA} = \frac{AS}{A0} \times \frac{1000}{W} \times \frac{1}{T} \times \frac{50}{1000} \mu\text{molNO}^{2-}\text{g}^{-1}\text{h}^{-1}$$

AS = Absorbance value of the solution
A0 = Absorbance value of the standard (0.0142)
W = Weight of fresh leaf samples
T = Time of incubation

Chlorophyll content measurement

Leaf chlorophyll content was measured before and after drought treatment (fifth months and sixth months in the main nursery) following the method outlined by Wellburn (1994). A total of 1 g oil palm leaves crushed with a mortar until pulverized, then added 20 mL acetone 80% and centrifuged to obtain a filtrate. The filtrate was collected in a 50 mL flask. Extraction was repeated by adding 3 mL acetone 80%, and then centrifuged again. The second filtrate was mixed with the first filtrate. Extraction of leaf dregs was carried out at least 3 times. The filtrate volume was adjusted by adding 80% acetone. The pigment extract in 80% acetone solvent was measured by UV-VIS spectrophotometer at wavelength 663 nm for chlorophyll a and 646 nm for chlorophyll b. The concentrations of chlorophyll a, b and total chlorophyll were calculated using the formula:

total chlorophyll (mg/g) = (17.30 x A_{646}) + (7.18 x A_{663}) ……..... 1)

chlorophyll a (mg/g) = (12.21 x A_{663}) + (2.81 x A_{646})……..... 2)

chlorophyll b (mg/g) = (20.13 x A_{646}) – (5.03 x A_{663})……..... 3)

where:
A_{646} = sample absorbance at 646 nm
A_{663} = sample absorbance at 663 nm

Stomatal observation

Stomatal closure was observed twice, at the age of fifth and sixth months of the seedlings, at the middle position of the leaf blade, the third leaf from the top of the canopy. The structure of stomatal anatomy and morphology for each treatment observed using Scanning Electron Microscope (SEM). The preparation consisted of three steps. The first step was fixation where the sample was immersed in 6% tannic acid solution overnight, then washed with cacodylate trihydrate, HCl and H_2O with pH 7.4 for 4 x 15 minutes. The second step was dehydration, the sample was soaked sequentially with 50% alcohol for 4 x 5 min, 70% alcohol for 20 min, and 85% alcohol for 20 min at 4ºC. Then, the sample was immersed in 95% alcohol for 20 min, absolute alcohol 2 x 10 min and t-butanol for 2 x 10 min at room temperature. The third step was gluing in which the sample of 0.5 x 0.5 cm size was taped to a sterile metal cylinder placed into an ion coating for vacuum, then coated with Pt-Au metal using an ion coating (Goldstein *et al*., 1992). The observations with SEM were photographed at 350x and 1500x magnifications (Talbot & Rosemary, 2013).

Data analysis

The data collected were then analyzed for the mean values obtained from ten replicates. Significantly different between the treatments and control seedlings were determined using the Analysis of Variance (ANOVA) at 5% level. If there were significantly different among the treatments it would be followed by the Duncan Multiple Range Test (DMRT) analysis.

Result and Discussion

Proline concentration, nitrate reductase activity (NRA), and chlorophyll a, b and total chlorophyll content, as well as stomatal closure, represent an analytical parameter to observe the physiological tolerance of oil palm seedlings with bio-Si treatments in a period of drought stress. In general, the results of the study showed that both solid and liquid bio-Si formula showed a positive effect on NRA, the content of chlorophyll a, b, and total chlorophyll and stomatal closure.

Proline content

Determination of free proline levels is a useful assay to monitor physiological status and to assess stress tolerance of higher plants (Ábrahám *et al*., 2010). Based on the data in this research, the untreated sample had a significantly different with solid and liquid bio-Si treatments, while the control (5 g NPK 15-15-15) had a significantly different only with liquid bio-Si treatments to proline concentration in the period of after drought stress. In Figure 1, the effect of solid bio-Si treatment on proline content indicates a lower proline accumulation than the untreated sample (blank) after drought stress. The lowest proline level of the solid bio-Si treatment (0.15 µmol/g) was found in 5 g NPK + 2.5 g bio-Si/seedling. While in Figure 2, all liquid bio-Si treatments had a lower proline accumulation compared to blank and control after drought stress. The lowest proline level of liquid bio-Si treatments was found in 5 g NPK 15-15-15 + 10^9 cfu SSM (0.05 µmol/g), followed by 5 g NPK 15-15-15 + 25 mL bio-Si/seedling (0.08 µmol/g). The increasing of proline level occurs when plants exposed to environmental stress, especially drought stress. Similarly, Cvikrová *et al.* (2013) reported that drought stress has increased proline levels in all genotypes. Proline has a function for organizing the osmotic degree of plant cells and so-called osmotic adjustment (Benny *et al*., 2015).

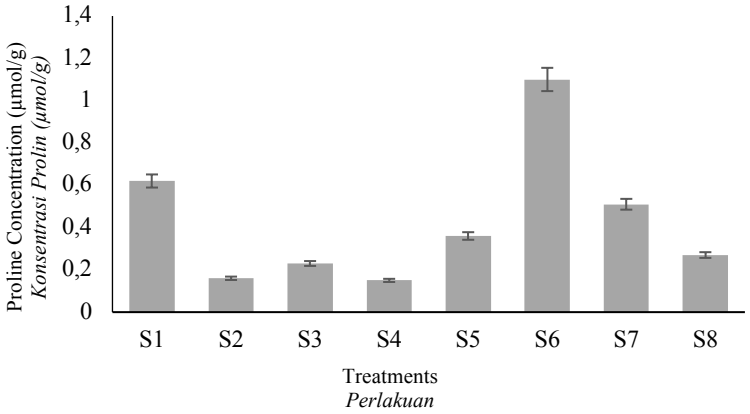

Figure 1. The effect of solid bio-Si on proline concentration in oil palm seedling after drought stress treatment. (S1) blank (without fertilizers), (S2) control (5 g NPK 15-15-15), (S3) 5 g NPK 15-15-15 + 10^9 cfu SSM, (S4) 5 g NPK 15-15-15 + 2.5 g bio-Si, (S5) 5 g NPK 15-15-15 + 5.0 g bio-Si, (S6) 5 g NPK 15-15-15 + 7.5 g bio-Si, (S7) 5 g NPK 15-15-15 + 10 g bio-Si, (S8) 5 g NPK 15-15-15 + 5 g Na_2SiO_3. Bar (I) is an error standard (ES).

Gambar 1. Pengaruh bio-Si padat terhadap kadar prolin pada bibit kelapa sawit setelah perlakuan cekaman kekeringan. (S1) blanko (tanpa pupuk), (S2) kontrol (5 g NPK 15-15-15), (S3) 5 g NPK + 10^9 cfu mikrob pelarut silika, (S4) 5 g NPK 15-15-15 + 2,5 g bio-Si, (S5)5 g NPK 15-15-15 + 5,0 g bio-Si, (S6)5 g NPK 15-15-15 + 7,5 g bio-Si, (S7) 5 g NPK 15-15-15 + 10 g bio-Si, (S8) 5 g NPK 15-15-15 + 5 g Na_2SiO_3. Tanda bar (I) adalah standar error (SE).

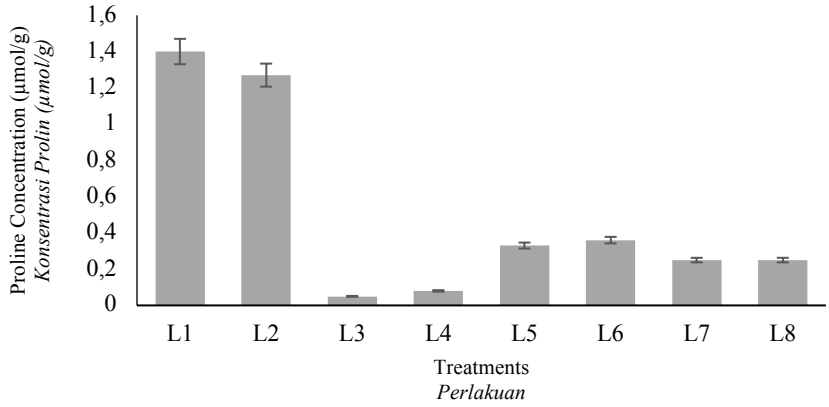

Figure 2. The effect of liquid bio-Si on proline concentration in oil palm seedling after drought stress treatment. (L1) blank (without fertilizers), (L2) control (5 g NPK 15-15-15), (L3) NPK + 10^9 cfu SSM, (L4) 5 g NPK 15-15-15 + 25 mL bio-Si, (L5) 5 g NPK 15-15-15 + 50 mL bio-Si, (L6) 5 g NPK 15-15-15 + 75 mL bio-Si, (L7) 5 g NPK 15-15-15 + 100 mL bio-Si, (L8) 5 g NPK 15-15-15 + 50 mL Na_2SiO_3. Bar (I) is an error standard (ES).

Gambar 2. Pengaruh bio-Si cair terhadap konsentrasi prolin pada bibit kelapa sawit setelah perlakuan cekaman kekeringan. (L1) blanko (tanpa pupuk), (L2) kontrol (5 g NPK 15-15-15), (L3)5 g NPK + 10^9 cfu mikrob pelarut silika, (L4) 5 g NPK 15-15-15 + 25 mL bio-Si, (L5) 5 g NPK 15-15-15 + 50 mL bio-Si, (L6)5 g NPK 15-15-15 + 75 mL bio-Si, (L7)5 g NPK 15-15-15 + 100 mL bio-Si, (L8) 5 g NPK 15-15-15 + 50 mL Na_2SiO_3. Tanda bar (I) adalah standar error (SE).

Osmotic adjustment is a mechanism during periods of water deficiency for maintenance of water potential in plant cells (Cvikrová *et al*., 2013). Si-solubilizing microbes are able to degrade silicates including aluminium silicates and render it available to plants (Santi *et al*., 2018). Several mechanisms of it are involving acidolysis, alkaline hydrolysis, ligand degradation, enzymolysis, capsule adsorption, extracellular polysaccharides and redox.

The action of microbes and chemical reactions in the soil make the silicon available to the plants. Santi *et al*. (2018) observed that deposition bio-silica occurs in endodermis part of roots oil palm seedling. Therefore bio-Si can enhance root tolerance to drought and promotes root growth

Merwad *et al*. (2018) reported the result of their study, that Si improve the growth criteria, yield characteristics, leaf chlorophyll content a and b, total carotenoids, shoots and nutrient seeds, relative leaf water content and membrane stability index, and further increasing the activity of mites and antioxidant enzyme activity of cowpea leaf. This indicates solid and liquid bio-Si formula plays an important role in keeping plants vigor and more tolerance with drought stress, so the proline level was smaller than that of in blank and control treatments. The result also suggests that solid and liquid bio-Si could replace proline in plants under drought stress, functioning as a major osmo-protectant. Pei *et al*. (2010) observed that proline concentration increases under drought stress and that Si addition decreases the proline accumulation in wheat leaves. It suggested Si-induced decrease in proline accumulation was a sign of stress injury alleviation.

Nitrate reductase activity (NRA)

Based on the analysis of NRA (µ mol NO^{2-}/g/hour) in this research, the solid and liquid bio-Si treated seedlings had a significantly different compared to both of blank and control in the period of before and after drought stress conditions. The optimum dosage of solid and liquid bio-Si treatments was increasing for NRA in the period of after drought stress condition (Figure 3 and 4). Normally, drought stress condition can affect the decreasing of NRA. However, application of 5 g NPK 15-15-15 + 7.5 g bio-Si, 5 g NPK 15-15-15 + 10^9 cfu SSM, and 5 g NPK 15-15-15 + 50 mL Na_2SiO_3 per seedling were increased for NRA values under drought stress conditions. Silicon has a positive impact on a plant by stimulating nutrient uptake and plant photosynthesis (Santi & Goenadi, 2017). The NRA was mainly affected by the presence of bio-Si which stimulating plants to absorb essential nutrients at optimal levels in plants tissue, especially the nitrate content.

The high of NRA activity of oil palm seedlings before and after drought stress with the solid bio-Si application were 3569.7 µ mol NO^{2-}/g/hour (5 g NPK 15-15-15 + 5.0 g bio-Si/seedling) and 7528.7 µ mol NO^{2-}/g/hour (5 g NPK 15-15-15 + 7.5 g bio-Si/seedling) respectively. Further, with liquid bio-Si applications were 1392.0 µ mol NO^{2-}/g/hour (5 g NPK 15-15-15 + 75 mL bio-Si/seedling) and 1277.7 µmoL NO^{2-}/g/hour (5 g NPK 15-15-15 + 50 mL bio-Si/seedling). The value of NRA indicates that enzyme activity plays a role in the nitrate assimilation process. Amir *et al.* (2015) observed that the occurrence of translocation process of N from the nodule to the entire plant increases the N content in plant tissues and the amount of NRA that enhances the increase of N absorption in Wilis soybean cultivar. In our research, were assumed that solid and liquid bio-Si involved in nitrate uptake, allocation, and storage in oil palm seedling.

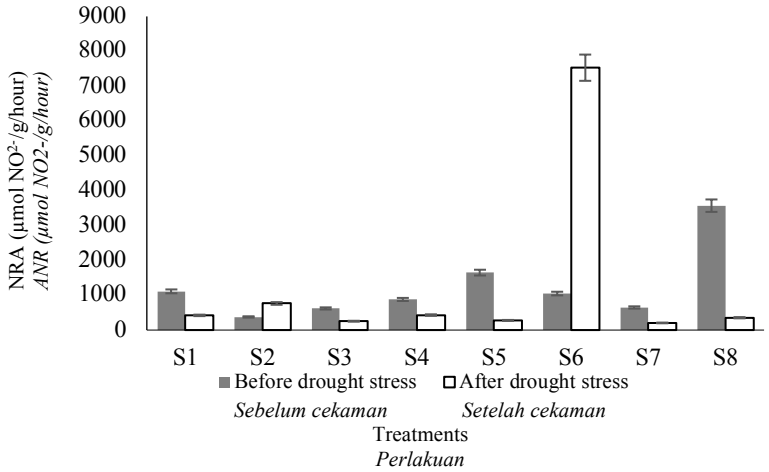

Figure 3. The effect of solid bio-Si on nitrate reductase activity in oil palm seedlings before and after drought stress treatment. (S1) blank (without fertilizers), (S2) control (5 g NPK 15-15-15), (S3) 5 g NPK 15-15-15 + 10^9 cfu SSM, (S4) 5 g NPK 15-15-15 + 2.5 g bio-Si, (S5) 5 g NPK 15-15-15 + 5.0 g bio-Si, (S6) 5 g NPK 15-15-15 + 7.5 g bio-Si, (S7) 5 g NPK 15-15-15 + 10 g bio-Si, (S8) 5 g NPK 15-15-15 + 5 g Na_2SiO_3. Bar (I) is an error standard (ES).

Gambar 3. Pengaruh bio-Si padat terhadap aktivitas nitrat reduktase sebelum dan setelah perlakuan cekaman kekeringan. (S1) blanko (tanpa pupuk), (S2) kontrol (5 g NPK 15-15-15), (S3) 5 g NPK + 10^9 cfu mikrob pelarut silika, (S4) 5 g NPK 15-15-15 + 2,5 g bio-Si, (S5)5 g NPK 15-15-15 + 5,0 g bio-Si, (S6)5 g NPK 15-15-15 + 7,5 g bio-Si, (S7) 5 g NPK 15-15-15 + 10 g bio-Si, (S8) 5 g NPK 15-15-15 + 5 g Na_2SiO_3. Tanda bar (I) adalah standar error (SE).

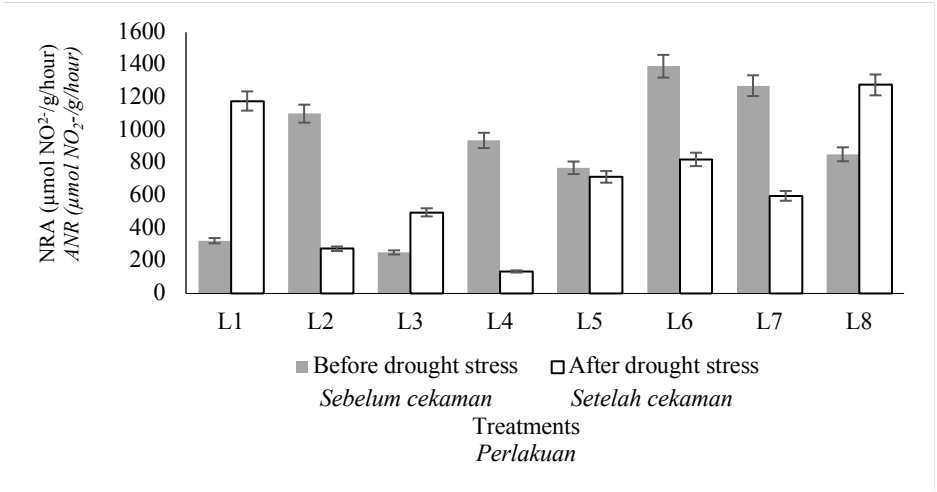

Figure 4. The effect of liquid bio-Si on nitrate reductase activity before and after drought stress. (L1) blank (without fertilizers), (L2) control (5 g NPK 15-15-15), (L3) NPK + 10^9 cfu SSM, (L4) 5 g NPK 15-15-15 + 25 mL bio-Si, (L5) 5 g NPK 15-15-15 + 50 mL bio-Si, (L6) 5 g NPK 15-15-15 + 75 mL bio-Si, (L7) 5 g NPK 15-15-15 + 100 mL bio-Si, (L8) 5 g NPK 15-15-15 + 50 mL Na_2SiO_3. Bar (I) is an error standard (ES).

Gambar 4. Pengaruh bio-Si cair terhadap aktivitas nitrat reduktase sebelum dan setelah cekaman kekeringan. (L1) blanko (tanpa pupuk), (L2) kontrol (5 g NPK 15-15-15), (L3) 5 g NPK + 10^9 cfu mikrob pelarut silika, (L4) 5 g NPK 15-15-15 + 25 mL bio-Si, (L5) 5 g NPK 15-15-15 + 50 mL bio-Si, (L6)5 g NPK 15-15-15 + 75 mL bio-Si, (L7)5 g NPK 15-15-15 + 100 mL bio-Si, (L8) 5 g NPK 15-15-15 + 50 mL Na_2SiO_3. Tanda bar (I) adalah standar error (SE).

Chlorophyll a, b and total chlorophyll

The measurement of chlorophyll content is one approach to study the effect of drought stress which is closely related to the rate of photosynthesis (Li *et al*., 2006). Based on the data analysis of chlorophyll a, b and total chlorophyll (Table 1), there are not significantly different between solid bio-Si, blank and control treatments before and after the drought stress. The highest content of chlorophyll a, b and total chlorophyll in the solid bio-Si were 31.6 chlorophyll a (5 g NPK 15-15-15 + 2.5 g bio-Si/seedling), 52.3 chlorophyll b and 50.2 total chlorophyll (5 g NPK 15-15-15 + 10^9 cfu SSM) that measured before drought stress. Further, the highest chlorophyll a, b and total chlorophyll were 32.1; 52.2; and 51.1 at 5 g NPK 15-15-15 + 2.5 g bio-Si/seedling after drought stress treatment. Some treatments had lower chlorophyll content than blank or control before drought stress, other treatments had similar chlorophyll content and even increase of chlorophyll content after drought stress, while the treatment 5 g NPK 15-15-15 + 10^9 cfu SSM decreased chlorophyll content after drought stress. It was suspected that the absence of bio-Si treatment and silicon was only obtained from the soil dissolved by SSM as a treatment. This showed that bio-Si tends to be able to increase chlorophyll content in drought stress condition. Koentjoro *et al*. (2017) also reports that the increasing application of silicon concentration tends to increase chlorophyll content.

Meanwhile, the liquid bio-Si treatment that had a significantly higher to the blank and control before and after drought stress was 5 g NPK 15-15-15 + 25 mL bio-Si/seedling on chlorophyll b and total chlorophyll content and 5 g NPK 15-15-15 + 50 mL bio-Si/seedling on chlorophyll b (Table 2). The maximum content of chlorophyll a, b and total chlorophyll in the liquid bio-Si treatment was 5 g NPK 15-15-15 + 25 mL bio-Si/seedling in the period before drought stress. However, in the period after drought stresses the maximum of chlorophyll a, b and total chlorophyll content was obtained by 5 g NPK 15-15-15 + 50 mL Na_2SiO_3/seedling. The mechanism of water deficit stress tolerance of silicon application i.e. improved photosynthesis and increased chlorophyll content to allow a constant supply of assimilates to the growing tissues (Ouzounidou *et al*., 2016). Further, Chen *et al*. (2011) reported that Si addition increases drought stress through the enhancement of photochemical efficiency and adjustment of the mineral nutrient absorption in rice plants.

Stomatal closure

The response of drought stress determined by the stomatal closing in the period before and after drought stress. Based on the SEM analysis, it can be shown that the solid and liquid bio-Si treatments had more numbers of stomatal openings than those found at the blank and control treatments (Figure 5 and 6). When beginning to drought stress, plants decrease the leaf water loss through stomatal closure for decreasing the leaf transpiration rate (Chen *et al*., 2018).

Table 1. The effect of solid bio-Si treated on chlorophyll a, b, and total chlorophyll content before and after drought stress

Tabel 1. Pengaruh bio-Si dalam bentuk padatan terhadap kandungan klorofil a, b dan total klorofil sebelum dan setelah cekaman kekeringan

Treatments (per seedling) *Perlakuan (per bibit)*	Before drought stress *Sebelum cekaman kekeringan*			After drought stress *Setelah cekaman kekeringan*		
	Chlorophyll a *Klorofil* a	Chlorophyll b *Klorofil* b	Total Chlorophyll *Klorofil* total	Chlorophyll a *Klorofil* a	Chlorophyll b *Klorofil* b	Total Chlorophyll *Klorofil* total
a. Blank (without fertilizers) *Blanko (tanpa pupuk)*	31.7 a*)	51.8 ab*)	45.8 ab*)	31.7 a	51.9 ab*)	50.4 a
b. Control (5 g NPK 15-15-15) *Kontrol (5 g NPK 15-15-15)*	31.8 a	51.6 ab	46.0 ab	27.7 a	48.5 ab	40.5 a
c. 5 g NPK 15-15-15 + 10^9 cfu SSM 5 g NPK 15-15-15 + *10^9 cfu MPS*	32.2 a	52.3 a	50.2 a	28.9 a	47.7 b	40.7 a
d. 5 g NPK 15-15-15 + 2.5 g bio-Si 5 g NPK 15-15-15 + *2,5 g bio-Si*	31.6 a	51.0 ab	46.2 ab	32.1 a	52.2 a	51.1 a
e. 5 g NPK 15-15-15 + 5.0 g bio-Si 5 g NPK 15-15-15 + *5,0 g bio-Si*	19.1 c	28.4 d	24.8 d	31.0 a	50.1 ab	47.2 a
f. 5 g NPK 15-15-15 + 7.5 g bio-Si 5 g NPK 15-15-15 + *7,5 g bio-Si*	27.3 ab	43.7 c	36.7 c	31.7 a	51.9 ab	50.2 a
g. 5 g NPK 15-15-15 + 10 g bio-Si 5 g NPK 15-15-15 + *10 g bio-Si*	26.1 b	43.1 c	35.1 c	32.1 a	52.5 a	47.3 a
h. 5 g NPK 15-15-15 + 5 g Na_2SiO_3 5 g NPK 15-15-15 + *5 g Na_2SiO_3*	30.0 ab	48.0 b	41.4 bc	31.1 a	50.0 ab	45.6 a
Coefficient of variation (%) *Koefiesien keragaman (%)*	6.8	3.4	7.9	7.0	3.4	10.3

**) Means in the same column followed by the same letters are not significantly different according to Duncan's multiple range test at α = 0.05.*

*) Angka dalam kolom yang sama diikuti oleh huruf yang sama berarti tidak berbeda nyata menurut uji jarak berganda Duncan pada α = 0,05.

Table 2. The effect of liquid bio-Si treated on chlorophyll a, b, and total chlorophyll before and after drought stress

Tabel 2. Pengaruh bio-Si dalam bentuk cairan terhadap kandungan klorofil a, b dan total klorofil pada sebelum dan setelah cekaman kekeringan

Treatments (per seedling) *Perlakuan (per bibit)*	Before drought stress *Sebelum cekaman kekeringan*			After drought stress *Setelah cekaman kekeringan*		
	Chlorophyll a *Klorofil* a	Chlorophyll b *Klorofil* b	Total Chlorophyll *Klorofil* total	Chlorophyll a *Klorofil* a	Chlorophyll b *Klorofil* b	Total Chlorophyll *Klorofil* total
a. Blank (without fertilizers) *Blanko (tanpa pupuk)*	29.7 a	49.3 ab*)	40.9 b*)	29.5 a	46.3 bc*)	41.4 ab
b. Control (5 g NPK 15-15-15) *Kontrol (*5 g NPK 15-15-15*)*	27.6 a	45.6 bc	37.2 c	26.9 a	40.1 d	37.6 b
c. 5 g NPK 15-15-15 + 10^9 cfu SSM 5 g NPK 15-15-15 + *10^9 cfu MPS*	26.9 a	42.5 c	35.9 c	28.8 a	47.6 abc	39.2 b
d. 5 g NPK 15-15-15 + 25 mL bio-Si 5 g NPK 15-15-15 + *25 mL bio-Si*	30.9 a	51.2 a	43.6 a	31.2 a	51.7 ab	44.5 b
e. 5 g NPK 15-15-15 + 50 mL bio-Si 5 g NPK 15-15-15 + *50 mL bio-Si*	27.4 a	43.2 c	37.1 c	29.0 a	47.3 abc	39.4 ab
f. 5 g NPK 15-15-15 + 75 mL bio-Si 5 g NPK 15-15-15 + *75 mL bio-Si*	29.6 a	47.9 ab	40.5 b	27.3 a	44.5 cd	36.7 b
g. 5 g NPK 15-15-15 + 100 mL bio-Si 5 g NPK 15-15-15 + *100 mL bio-Si*	30.0 a	49.2 ab	41.3 ab	30.9 a	49.7 abc	43.1 ab
h. 5 g NPK 15-15-15 + 50 mL Na_2SiO_3 5 g NPK 15-15-15 + *50 mL Na_2SiO_3*	29.2 a	45.2 bc	41.4 ab	32.1 a	52.5 a	51.8 a
Coefficient of variation (%) *Koefisien keragaman (%)*	7.2	3.6	10.3	9.2	4.6	11.9

**) Means in the same column followed by the same letters are not significantly different according to Duncan's multiple range test at α = 0.05.*

*) Angka dalam kolom yang sama diikuti oleh huruf yang sama berarti tidak berbeda nyata menurut uji jarak berganda Duncan pada α = 0,05.

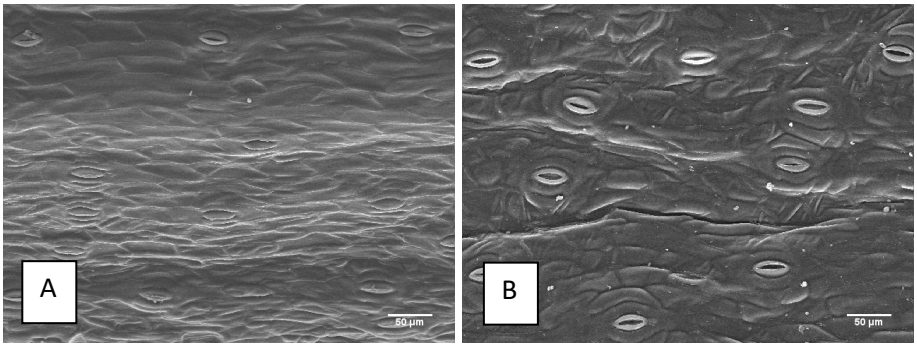

Figure 5. Scanning electron microscopy of oil palm leaf surfaces showing open stomata in application Na_2SiO_3 and solid bio-Si and closing stomata in treatment without application bio-Si in the period after drought stress treatment. (A) closing stomata in application of 5 g NPK 15-15-15 and (B) opening stomata in application of 5 g NPK 15-15-15 + 5 g bio-Si.

Gambar 5. Scanning electron microscopy *permukaan daun kelapa sawit menunjukkan pembukaan stomata pada aplikasi Na_2SiO_3 dan bio-Si padat dan penutupan stomata pada perlakuan tanpa aplikasi bio-Si padat setelah diberikan perlakuan cekaman kekeringan. (A) penutupan stomata pada aplikasi 5 g NPK 15-15-15 dan (B) pembukaan stomata pada aplikasi 5 g NPK 15-15-15 + 5 g bio-Si.*

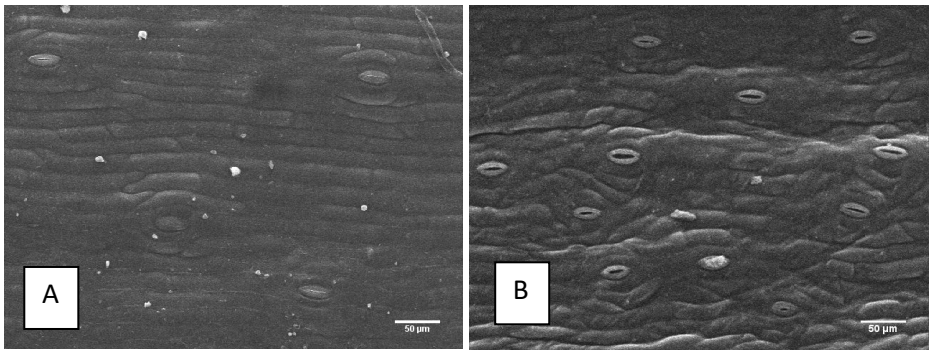

Figure 6. Scanning electron microscopy of oil palm leaf surfaces showing open stomata in application Na2SiO3 and liquid bio-Si and closing stomata in treatment without application bio-Si in the period after drought stress treatment. (A) closing stomata in application of 5 g NPK 15-15-15 and (B) opening stomata in application of 5 g NPK 15-15-15 + 25 mL bio-Si.

Gambar 6. Scanning electron microscopy *permukaan daun kelapa sawit menunjukkan pembukaan stomata pada aplikasi Na_2SiO_3 dan bio-Si cair dan penutupan stomata pada perlakuan tanpa aplikasi bio-Si cair setelah diberikan perlakuan cekaman kekeringan. (A) penutupan stomata pada aplikasi* 5 g NPK 15-15-15 *dan (B) pembukaan stomata pada aplikasi 5 g NPK 15-15-15 + 25 mL bio-Si.*

Silicon has an important role in the maintenance of plant mineral balance in drought-stressed plants (Zhu & Gong, 2014). Si as silicic acid [$Si(OH)_4$] that plant absorbed by root's cells is deposited into roots epidermal cells (Santi *et al*., 2018). Si can improve water status with reducing water loss in the leaf of a plant so that plants did not get drought stressed, turgor cover cells of the leaf were tending stabled, and stomatal still opened. When the cover cell of leaf takes water through osmosis, that will swell and become more turgid (Haryanti & Meirina, 2009). Decreasing stomatal opening and inhibition of chloroplast activity may cause decreased plant photosynthesis activity (Sokoto & Muhammad, 2014).

Conclusions

The influence of drought stress on plants can be determined by the response of plant physiology in addition to other vegetative growth parameters. Applications of solid and liquid bio-Si gave a positive response to the physiological of oil palm seedling to the drought stress. Application of solid bio-Si could enhance nitric reductase activity, chlorophyll a, b and total chlorophyll content before drought stress. After drought stress, the solid bio-Si formula application has nitrate reductase activity higher than blank and control. On the other hand, the liquid bio-Si application has higher nitrate reductase activity and chlorophyll b than that of blank and control before and after drought stress treatment. Physiological of oil palm seedlings to drought stress could be enhanced by the recommended dosage of 5 g NPK 15-15-15 + 7.5 g solid or 5 g NPK 15-15-15 + 75 mL liquid bio-Si/seedling.

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