Physiological Responses of Oil Palm Seedlings to the Drought Stress Using Boron and Silicon Applications

E.T.S. Putra, Issukindarsyah, Taryono and B.H. Purwanto
Faculty of Agriculture, Universitas Gadjah Mada, Bulaksumur, Yogyakarta, 55281, Indonesia

ABSTRACT
The objectives of the study were to determine (1) The level of physiological resistance of oil palm seedlings to drought stress by boron (B) and silicon (Si) application and (2) The mechanism of B and Si actions to induce physiological resistance of oil palm seedlings to drought stress. The B and Si were the elements capably inducing the internal resistance of plant tissues to drought stress, especially through physiological resistance mechanisms. Field trial was arranged in the factorial Randomized Complete Block Design (RCBD) using three blocks as replications. The first factor was six dose of B: 0.00, 0.17, 0.44, 0.87 and 1.31 g plant$^{-1}$. The second factor was five dose of Si: 0.00, 1.15, 2.31, 3.46 and 4.69 g plant$^{-1}$. Observations were done on the Nitrate Reductase Activity (NRA), the content of chlorophyll a, b and total, density, length and width of stomatal aperture, stomatal conductance and transpiration rate, photosynthetic rate and photosynthetic activity per plant, dry weight of plant parts and trunk height and diameter of the oil palm seedlings. The data were analyzed using ANOVA and the means were separated using Duncan’s multiple range test at 5% level. Meanwhile, the optimum dose of B and Si were determined using regression analysis. The results showed that B and Si application could induce physiological resistance of oil palm seedlings to drought stress. Mechanism of action of B in inducing physiological resistance of oil palm seedlings to drought stress were by increasing of greenish leaves, width of stomatal aperture and photosynthetic activity per plant while Si application capable to increase of greenish leaves and to decrease the density of lower leaf surface stomatal. The optimal dose of B was 0.33-0.57 g/seedlings and the optimal dose of Si was 2.22 g/seedling in inducing physiological resistance of oil palm seedlings to drought stress.

Key words: Oil palm, B and Si, physiological resistance, drought

INTRODUCTION
Oil palm responses to drought stress are generally negative. Several studies indicate that the drought lower the yield of fresh fruit bunches up to 26.30% (Al-Amin et al., 2011). The decline of oil palm yield is a direct effect of inhibition of photosynthetic rate of oil palm. Drought causes stomatal conductance of oil palm leaves decline rapidly because the stomata tend to close. The low stomatal conductances decrease the rate of CO$_2$ diffusion, so that the photosynthetic activity of oil palm is inhibited (Henson and Harun, 2007). Other factors that inhibit the rate of photosynthesis in drought conditions are the relative water content (Fahramand et al., 2014; Zain et al., 2014), chlorophyll a/b, the content of nitrogen and phosphorus in leaf tissue of plants (Legros et al., 2009; Cha-um et al., 2010; Cao et al., 2011; Son et al., 2011; Zlatev and Lindon, 2012; Ashraf and Harris, 2013) which are low.

The information in the previous paragraph suggests that oil palm is a commodity that is quite sensitive to drought. The situation is urgent to overcome because in the next few years the drought stress becomes more frequent with increasing the
intensity due to the phenomenon of global climate change, particularly in the area of oil palm plantations. Agronomic approaches can be taken to reduce the sensitivity of oil palm to drought. The approach is intended to stimulate the appearance of changes in the physiological characteristics of oil palm tissue and these changes are expected to induce the physiological resistance of oil palm to drought stress.

Boron (B) is an essential nutrient needed by plants to grow normally (Shaaban et al., 2004, 2006; Halder et al., 2007; Hajiboland and Farhanghi, 2010; Shaaban, 2010; Demiray and Dereboylu, 2013; Gupta and Solanki, 2013). Some studies indicate that B deficiency decreases the photosynthetic capacity of plant (Ahmed et al., 2008; Han et al., 2008; Ahmed et al., 2009; Hajiboland and Farhanghi, 2010; Hajiboland et al., 2012). The decline in photosynthetic capacity due to the low intracellular concentration of CO₂, chlorophyll degradation, inhibition of electron transfer processes and photo phosphorylation, structural and functional damage of leaves and the accumulation of sucrose and hexoses in leaves (Hajiboland and Farhanghi, 2010; Putra, 2010; Putra et al., 2010a, b, 2012; Hajiboland et al., 2012; Demiray and Dereboylu, 2013; Gupta and Solanki, 2013). These characteristics indicate that the plants experience oxidative stress. It can be concluded that B deficiency causes plants experience oxidative stress as in plants experiencing drought stress condition. This knowledge opens the opportunity to overcome the negative effects of drought through the application of B.

Silicon (Si) is not an essential nutrient for plants but it gives positive influence on the growth and development of plants, especially plants exposed to drought (Almeida et al., 2009; Gorecki and Danielski-Busch, 2009; Sacala, 2009; Tale Ahmad and Haddad, 2011; Bocharnikova and Benes, 2011; Son et al., 2011). Some studies indicate that Si application is able to avoid the damage of plant when grown under drought stress condition. These conditions indicate that the application of Si increases plant resistance to drought (Henriet et al., 2006, 2008; Sacala, 2009; Tale Ahmad and Haddad, 2011). The Si application helps the plants exposed to drought to maintain the balance of water level in the tissue, to increase photosynthetic activity (Biglary et al., 2011; Dastan et al., 2011; Rahimi et al., 2012; Bharwanana et al., 2013), to support the establishment of leaves, to maintain the structure of xylem vessels under conditions of rapid transpiration rate, to improve the balance of nutrients, to reduce minerals toxicity and to increase the mechanical strength of plant tissues (Hattori et al., 2005; Henriet et al., 2008; Ahmed et al., 2009; Sacala, 2009; Tale Ahmad and Haddad, 2011). Other mechanisms which are not less important in relation to the role of Si for resistance to drought is increased water use efficiency by reducing the rate of water loss. The rate of water loss decreases due to the application of Si is able to eliminate cuticular transpiration and at the same time to increase in the CO₂ assimilation rate and stomatal conductance (Gong et al., 2005; Hou et al., 2006; Sacala, 2009; Putra, 2010; Putra et al., 2010a, b; Tale Ahmad and Haddad, 2011; Gong, 2011).

Exploration on some previous paragraphs provides an overview of the potential emergence of physiological resistance of oil palm to drought stress through the application of B and Si. Nevertheless, accurate information in this regard up to now can not be found. Mechanism of action of B and Si in the inducing of physiological resistance of oil palm to drought also remains unclear. Therefore, a study that aimed to determine (1) The level of physiological resistance of oil palm to drought after applied using some doses of B and Si as well as, (2) Mechanism of action of B and Si in the induction of oil palm physiological resistance to drought was important to be done.

MATERIALS AND METHODS

Planting materials used in the study were five months old of oil palm seedlings, ready to be moved from pre-nursery to the main-nursery. The seed used were uniform in terms of the size of the seeds, healthy and comes from the same progeny. Seedlings planted in the polybags, 40×40 cm in size, were filled with top soil surface layer. Seeds were arranged at a spacing of 90×90×90 cm with equilateral triangles pattern.

Experiment was arranged in a Randomized Complete Block Design (RCBD) factorial with three blocks as replications. The first factor was the dose of boron (B) and the second factor was the dose of silicon (Si). The boric acid (H₃BO₃) as a source of B and sodium silicate (Na₂SiO₃) as a source of Si. The first factor consisted of six dose levels, namely 0.00, 0.17, 0.44, 0.87, 1.31 and 1.75 g/seedling. The second factor consists of five levels, namely 0.00, 1.15, 2.31, 3.46 and 4.69 g/seedling. Application of B and Si were done before drought stress treatment until four months after transplanting, once a month with the same dose every month depending on the treatment. The applications of B and Si were done by sowing in a circular array, about 10 cm from the base of the stem and then covered with soil.

Plants were also fertilized using NPK fertilizer (15:15:15), KCl and Kieserit. NPK fertilizer doses at 1st, 2nd, 3rd and 4th months after transplanting were 24.00, 28.00, 32.00 and 40.00 g/seedling, respectively. The KCl doses at 1st, 2nd, 3rd and 4th months after transplanting were 2.50, 2.83, 3.25 and 4.17 g/seedling, respectively. While, the application dose of kieserit at 1st, 2nd, 3rd and 4th months after transplanting were 10.81, 12.69, 13.07 and 3.85 g/seedling, respectively. In the first factor consisted of six dose levels.
moisture content of the soil at seedling media reached permanent wilting point. To anticipate the possibility of rain during the test, the seedlings being tested were placed in a plastic house. Matius et al. (2004) provide the information that the oil palm seedlings with the age of 14 months after transplanting, with the planting medium combination of soil: sand: compost (1:1:1), reached permanent wilting point after 18 days of drought stress treated with soil moisture content of approximately 10%.

The Nitrate Reductase Activity (NRA) was observed at five and six months after transplanting or before and after drought stress treatment. A total of 200 mg of oil palm leaves was sliced with a thickness of 1 mm and put into a black plastic tube that had been filled with a solution of 0.1 M Phosphate Buffer pH 7.5 by 5 mL. After 24 h the solution was replaced with the same volume and added 0.1 mL of 0.05 M NaNO₃ solution and then incubated for 2 h in the dark at room temperature. After incubation, 0.1 mL of solution was put into a test tube which had previously been filled with a solution of 1% amide sulfanil about 0.2 mL and 0.2 mL of a solution of N-naphthyl ethylene diamide 0.02%. After 10-15 min the color of the solution turned pink and was added 2.5 mL of distilled water so that the volume to 3 mL and shaken. Observations were made with a spectrophotometer at a wavelength of 540 nm. The NRA was expressed in a number of NO₂⁻ μmol g⁻¹ h⁻¹ by the equation:

\[ \text{NRA} = \frac{\text{AS}}{\text{A0}} \times \frac{1}{\text{W}} \times \frac{1}{\text{T}} \times \frac{500}{1000} \mu\text{mol NO}_2^- \text{ g}^{-1} \text{ h}^{-1} \]

\( \text{AS} = \text{Absorbance value of the solution} \)
\( \text{A0} = \text{Absorbance value of the standard (0.0106 or 0.0142)} \)
\( \text{B} = \text{Weight of fresh leaf samples} \)
\( \text{T} = \text{Time of incubation} \)

Leaf chlorophyll content and greenness was measured at five and six months after transplanting or before and after drought treatment. Leaf chlorophyll content was determined using the method that has been developed by Comb et al. (1985), known as Comb’s method while leaf greenness was measured using a SPAD 502. A total of 1 g oil palm leaves crushed with a mortar until pulverized, then added 20 mL of acetone 80% and filtered with Whatman paper No. 1. Solution absorbance was read using Spectronic 21D Milton Roy at a wavelength of 645 and 663 μm. The concentration of chlorophyll a and b was calculated using the formula:

- Chlorophyll a = 0.0127×A663−0.00269×A645
- Chlorophyll b = 0.0229×A645−0.00468×A663
- Total chlorophyll concentration = concentration of chlorophyll a+b
- A645 = Absorbance at a wavelength of 645 μm
- A663 = Absorbance at a wavelength of 663 μm

Components of stomatal morphology observed included stomatal density, length and width of stomatal aperture. Sampling was done twice, at the age of five and six months after transplanting. The observation was done in the middle of the leaf blade, leaf number 2 or 3 from the top of canopy. The collection of stomatal mold was done by applying clear nail polish to the leaf surface. After dry, nail polish was removed by attaching a transparent tape on the glass object and observed using a microscope. Stomatal number was observed using ocular that completed with net micrometer, 100X magnifications. Stomatal aperture width was observed using optilab tool, with 420X magnification of micrometer (Indradewa et al., 2002).

Physiological variables were included stomatal conductance (Photosynthetic Analyzer type LI Cor LI 6400), transpiration rate (Photosynthetic Analyzer type LI Cor LI 6400) and photosynthesis (Photosynthetic Analyzer type LI Cor LI 6400). Physiological activities of the plant were measured twice at 5 and 6 months after transplanting or before and after drought treatment.

Plant dry weight was measured at 5 and 6 months after transplanting or before and after drought treatment. Plant dry weight separated into any part of the roots, stems and leaves. Dry weights were obtained by dried the samples using oven at 65°C for 48 h until the weight was constant. Samples were then weighed using digital balances.

Trunk height and diameter of oil palm seedlings were observed every week, start at one week after transplanting. The tools used were ruler and digital calipers. Trunk height was measured from the base of the stem above the soil surface to the tip of the highest leaf. While the trunk diameter measured at a height of ±2 cm above the soil surface.

The data were analyzed using the Analysis of Variance (ANOVA) at 5% level and followed by Duncan Multiple Range Test (DMRT) if there were significant differences among the treatments. The optimum doses of B and Si that were able to induce physiological resistance of oil palm seedlings to drought stress were determined using regression analysis. Meanwhile, the relationship patterns between the parameters were determined using correlation analysis. All the analyses were performed using the General Linear Model Procedure (PROC GLM) (SAS Institute Inc., 1990).

**RESULTS**

Dose of B and Si have no regression relationship with NRA, in the period of before and after drought stress treatment (Table 1 and 2). Any dose of B and Si were applied to the oil palm seedlings were not able to influence the NRA in the leaf tissue. Based on the available data it can be said that the role of B and Si in the process of induction of resistance of oil palm seedlings to drought stress was not through strengthening the capacity of NRA. Nitrate reductase activity can not be used as an indicator to detect the capacity of oil palm seedlings to counter the negative influence of drought stress after getting the application of B and Si.

Application dose of B did not have regression relationships with chlorophyll content, particularly in the
Chlorophyll a (mL g⁻¹)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before drought stress</th>
<th>Note</th>
<th>After drought stress</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRA (µmol/NO₂⁻ h)</td>
<td>y = 0.36x+14.36</td>
<td>ns</td>
<td>y = -1.11x +35.4</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>y = 4.19x²-6.85x+15.80</td>
<td>ns</td>
<td>y = 0.09 x²-1.11x +35.40</td>
<td>ns</td>
</tr>
</tbody>
</table>

ns: No regression

Table 2: Regression between application dose of Si and NRA

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before drought stress</th>
<th>Note</th>
<th>After drought stress</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRA (µmol/NO₂⁻ /jam)</td>
<td>y = 0.56x+13.35</td>
<td>ns</td>
<td>y = 1.77x+29.6</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>y = 0.74x²-2.85x+15.31</td>
<td>ns</td>
<td>y = 1.55x²-5.38x+33.72</td>
<td>ns</td>
</tr>
</tbody>
</table>

ns: No regression

Table 3: Regression between application doses of B with content of chlorophyll a, b and total and also leaf greenish

<table>
<thead>
<tr>
<th>Variables</th>
<th>Before drought stress</th>
<th>Note</th>
<th>After drought stress</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll a (mL g⁻¹)</td>
<td>y = -0.01x+0.43</td>
<td>ns</td>
<td>y = 0.003x+0.40</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>y = 0.004x²+0.02x+0.43</td>
<td>ns</td>
<td>y = -0.04x²+0.07x+0.39</td>
<td>ns</td>
</tr>
<tr>
<td>Chlorophyll b (mL g⁻¹)</td>
<td>y = -0.01x+0.586</td>
<td>ns</td>
<td>y = 0.010x+0.513</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>y = 0.002x²-0.019x+0.587</td>
<td>ns</td>
<td>y = 0.07x²+0.14x+0.49</td>
<td>ns</td>
</tr>
<tr>
<td>Chlorophyll a/b</td>
<td>y = 0.001x+0.730</td>
<td>ns</td>
<td>y = 0.000x+0.807</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>y = 0.02x²-0.03x+0.74</td>
<td>ns</td>
<td>y = 0.082x²-0.14x+0.84</td>
<td>ns</td>
</tr>
<tr>
<td>Total chlorophyll (mL g⁻¹)</td>
<td>y = -0.03x²+0.04x+1.01</td>
<td>*</td>
<td>y = 0.013x+0.913</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>y = -0.09x²+0.18x+0.88</td>
<td>ns</td>
<td>y = 0.199x+4.61</td>
<td>ns</td>
</tr>
<tr>
<td>Leaf greenish</td>
<td>y = -2.09x+53.95</td>
<td>ns</td>
<td>y = -2.03x²+2.30x+40.92</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>y = 1.26x² - 4.26x+54.38</td>
<td>ns</td>
<td>y = -0.01x+0.586</td>
<td>ns</td>
</tr>
</tbody>
</table>

*: Regression, ns: No regression

period after drought stress (Table 3). Nevertheless, different things found in leaf greenness variables. In the period after drought stress, application dose of B had a quadratic regression relationship with leaves greenness. Leaf chlorophyll content was closely related to NRA, because the rate of synthesis depends on the presence of NRA. Since NRA was not affected by B application dose (Table 1), the same trend found in variable of chlorophyll content of leaves. The absence of regression relationship between application dose of B with leaf chlorophyll content was determined by the presence of NRA in the period of after drought stress. In the period before the drought stress treatment application dose of B had quadratic regression relationship with the total chlorophyll content, total chlorophyll content which reached a maximum at 0.67 g of B/seedling. However, the relationship then disappears when the oil palm seedlings exposed to drought stress. In the period after drought stress treatment, the greenness of leaves was affected by B application doses though chlorophyll content of leaves was not. Leaf greenness of oil palm seedlings after drought stress period reaches a maximum at 0.57 g of B/seedling. The increase in dose until 0.57 g of B/seedling was always followed by the increase in leaf greenness, however, after 0.57 g of B/seedling leaf greenness level was decreasing due to the toxicity of B. Based on the available data it can be stated that the chlorophyll content of leaves was more sensitive to drought stress when compared to green leaves. Chlorophyll content of leaves before the drought stress treatment that was influenced by dose of B quickly turned into not affected by the dose of B under drought stress while leaves greenness precisely had the opposite effect with leaf chlorophyll content (Table 3).

The dose of Si does not have regression relationship with chlorophyll a, b and total, the ratio of chlorophyll a/b and the leaves greenness of oil palm seedlings, in the period before the drought stress treatment (Table 4). In the period after drought stress treatment, the dose of Si had quadratic regression relationship with the ratio of chlorophyll a/b and leaf greenish of oil palm seedlings (Table 4). The ratio of chlorophyll a/b reaches a maximum at 0.67 g of Si/seedling. At the application dose of Si >0.67 g/seedling, increasing the dose of Si would lower ratio of chlorophyll a/b because chlorophyll a degraded due to Si toxicity. The increase in dose of Si in the range of 0-0.67 g/seedling could increase the ratio of chlorophyll a/b due to the presence of Si dose stimulated the synthesis of chlorophyll a in one hand while on the other hand chlorophyll b decreased. The application of the proper dosage of Si helped plants to absorb essential nutrients and prevent poisoning of a nutrient, consequently the presence of all the essential nutrients in plant tissues at optimal levels. Optimal levels of nutrients in plant tissue stimulated the plant to synthesize chlorophyll a which was one of the pigments in the chloroplast that fills the complex reaction center as accumulator of the solar radiation energy that had been captured by chlorophyll b. While the content of chlorophyll b in leaf tissue declined quite dramatically due to drought stress. Chlorophyll b is a pigment that is more sensitive to damage when compared to chlorophyll a, under drought stress condition.
Table 4: Regression between dose of Si with chlorophyll a, b and total content, chlorophyll a/b and also leaf greenness

<table>
<thead>
<tr>
<th>Variables</th>
<th>Before drought stress</th>
<th>Note</th>
<th>After drought stress</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll a (mL g⁻¹)</td>
<td>y = -0.001x+0.42</td>
<td>ns</td>
<td>y = 0.001x+0.4</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>y = -0.001x²+0.0003x+0.42</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorophyll b (mL g⁻¹)</td>
<td>y = 0.003x+0.57</td>
<td>ns</td>
<td>y = -0.001x+0.52</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>y = -0.002x²-0.01x+0.57</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorophyll a/b</td>
<td>y = -0.01x+0.75</td>
<td>ns</td>
<td>y = 0.01x²-0.03x+0.83</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>y = -0.003x²-0.01x+0.75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total chlorophyll (mL g⁻¹)</td>
<td>y = 0.001x+0.99</td>
<td>ns</td>
<td>y = 0.003x+0.92</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>y = 0.001x+0.003x+0.99</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf greenness</td>
<td>y = -0.54x+53.60</td>
<td>ns</td>
<td>y = -0.74x²+3.29x+39.01</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>y = -0.002x²-0.53x+53.60</td>
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<td></td>
</tr>
</tbody>
</table>

*: Regression, ns: No regression

Table 5: Regression between application dose of B with density, width and length of upper the leaf surface of stomata

<table>
<thead>
<tr>
<th>Variables</th>
<th>Before drought stress</th>
<th>Note</th>
<th>After drought stress</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomatal density</td>
<td>y = -0.01x+6.18</td>
<td>ns</td>
<td>y = 0.09x+4.53</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>y = 0.49x²-0.85x+6.35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomatal width (µm)</td>
<td>y = -0.09x+6.40</td>
<td>ns</td>
<td>y = 0.04x+4.87</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>y = -0.17x²+0.19x+6.35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomatal length (µm)</td>
<td>y = 0.09x+17.52</td>
<td>ns</td>
<td>y = 0.15x+16.83</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>y = 0.64x²-1.02x+17.74</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ns: No regression</td>
<td></td>
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</tr>
</tbody>
</table>

Table 6: Regression between application dose of B with density, width and length of below the leaf surface of stomata

<table>
<thead>
<tr>
<th>Variables</th>
<th>Before drought stress</th>
<th>Note</th>
<th>After drought stress</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomatal density</td>
<td>y = -0.42x+29.01</td>
<td>ns</td>
<td>y = -0.42x+26.18</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>y = 0.93x²-2.02x+29.33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomatal width (µm)</td>
<td>y = 0.04x+6.07</td>
<td>ns</td>
<td>y = 0.19x+4.89</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>y = 0.37x²-0.61x+6.19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomatal length (µm)</td>
<td>y = -0.06x+19.05</td>
<td>ns</td>
<td>y = 0.19x+18.33</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>y = 0.27x²-0.52x+19.14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ns: No regression</td>
<td></td>
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<td></td>
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</tbody>
</table>

*: Regression, ns: No regression

Leaf greenness of oil palm seedlings after drought stress period has quadratic regression relationship with the dose of Si application. The leaf greenness of oil palm seedlings reaches a maximum at 2.22 g of Si/seedling, in the period after drought stress treatment. Up to 2.22 g of Si/seedling, the increase in the dose of Si was always followed by an increase in leaf greenness of oil palm seedlings. The rise of leaf greenness level was caused by the increase in chlorophyll a and ratio of chlorophyll a/b and also the decrease of chlorophyll b. The higher level of ratio of chlorophyll a/b caused oil palm leaves look greener. The green color came primarily from the accumulation of chlorophyll a.

Morphological characterizations of stomata include stomatal density and width and length of stomatal openings variables. The samples were stomata that located at the top or bottom surface of the leaf of oil palm seedlings. Application dose of B did not have regression relationship with all stomatal morphology, particularly the stomata were located on the upper surface of the leaves in the period of before and after drought stress treatment (Table 5). The same condition was found in the stomata that located below the surface of the leaf of oil palm seedlings, where the dose of B did not have regression relationship with all the morphological characteristics of the stomata in the period of before the drought stress treatment (Table 6). In the period after drought stress treatment, the density and length of the stomata that located in the below of the leaf surface of oil palm seedlings also did not have regression relationship with application dose of B. However, the application dose of B had linear regression relationship with the width of stomatal opening located below the leaf surface in the period of after drought stress treatment (Table 6). Based on the available data it can be concluded that most of the morphological characters of the stomata were controlled by the genetic of the plant and does not change with the agronomic treatments, such as B applications. The stomatal morphological characters that can change with the agronomic treatment such as B applications were the width of stomatal opening. The width of the stomatal openings located below the leaf surface of oil palm seedlings in the period after drought stress treatment continued to increase in line with the increase of B application dose up to 1.75 g/seedling.

In the period before the drought stress treatment, application dose of B did not give significant effect on all the morphological characters of the stomata. Throughout this experiment, the planting medium having sufficient soil moisture so that the oil palm seedlings can absorb water
sufficiently. The sufficient of water content in the plant tissue, especially leaf organ, stimulated the increase of length and width of stomatal opening, therefore the effect of the application of B on stomatal morphological characters became insignificant. In the period after drought stress treatment, application of B on oil palm seedlings were able to increase the translocation of assimilates from leaf mesophyll to others organs, one of them was guard cells which were located on the leaf surface. The higher of assimilate concentration in the leaf surface. The higher of assimilate concentration in the guard cells could lower the water potential in that cells to become much lower compared to other cells in the leaf surface. As a result, the movement of water molecules from the outside into the guard cells would occur. Guard cells experienced a full turgor, resulting in wider stomatal opening, although no change on the density and length of the stomata. The increases in width of stomatal opening stimulate an increase in stomatal conductance. So it is clear that the application of B stimulated the increase in stomatal conductance directly at the time of oil palm seedlings exposed to drought stress. Application of Si did not have a regression relationship with all the stomatal morphological characters located on the upper leaf surface of oil palm seedlings, in the period before and after drought stress treatment (Table 7). All stomatal morphological characters located below the leaf surface of oil palm seedlings also did not have a regression relationship with the dose of Si in the period before the application of drought stress treatment (Table 8). The Si application also did not have a regression relationship with the width and length of stomatal opening that were located below the surface of oil palm seedlings, in the period of after drought stress treatment.

The application dose of Si had a linear regression relationship with the bottom surface of the stomatal density of leaves of oil palm seedlings (Table 8). The increase in the dose of Si application up to 4.61 g/seedling was always followed by the decrease in stomatal density at below surface of oil palm seedlings leaves in the period of after drought stress treatment. The contribution of Si in the process of induction of oil palm seedlings resistance to drought stress had different mechanisms with B. Mechanism adopted by Si to improve the resistance of oil palm seedlings to drought stress through the transpiration activity suppression. Transpiration activity suppressed by reducing stomatal density and stomatal density decreases while as induced by Si. The increase of Si in the leaf tissue inhibits the rate of modification of epidermal cells into the guard cells so that the numbers of guard cells were much reduced. Reducing the number of guard cells automatically reduced the number of holes as a result of stomatal density per unit leaf area became lower. The B and Si applications had no regression relationship with stomatal conductance and transpiration rate of oil palm seedlings in the period of before and after drought stress treatment (Table 9 and 10). Stomatal conductance and transpiration rate of oil palm seedlings were applied with B and Si were similar to seedlings that do not applied with B and Si. The increase in dose application of B up to 1.75 g/seedlings was able to increase the width of stomatal opening of oil palm
Application of B only affected the photosynthetic activity per plant but did not affect the rate of photosynthesis of oil palm seedlings (Table 11), after a period of drought stress. In the period after drought stress, photosynthetic activity per plant reached a maximum at 0.35 g B/seedling. Increasing doses of B >0.21 g/seedling inhibits the activity of photosynthesis per plant. Inhibition was associated with a decrease in total chlorophyll content due to the damaged of chlorophyll caused by B toxicity at doses >0.21 g/seedling. 

The rate of photosynthesis and photosynthetic activity per plant of oil palm seedlings after drought stress treatment were lower when compared to the period before drought stress period. The decline was caused by the limited availability of water, CO2 and chlorophyll degradation in the period of after drought stress.

Application of Si did not have a regression relationship with photosynthetic rate and photosynthetic activity per plant, before and after the oil palm seedlings exposed to drought stress (Table 12). The rate of photosynthesis and photosynthetic activity per plant had equal value at varying doses of Si up to 4.61 g/seedling. However, it was not known how the relationship between the dose of Si with photosynthetic activity per plant at doses >4.61 g/seedling. Therefore, further research on the application of Si at a dose of >4.61 g/seedling needs to be conducted.

The dry weight of leaves, stems, roots and total of oil palm seedlings had a quadratic regression relationship with the dose of B but not so with the root/shoot (Table 13). Dry weight of leaves, stems, roots and total of oil palm seedlings reached a maximum at 0.35-0.70 g B/seedling, before the drought stress period. Increasing doses of B application >0.70 g/seedling lowered dry weight of leaves, stems, roots and total of oil palm seedlings, because of the availability of B in the soil beyond the requirement of oil palm seedlings. In the period following exposure to drought stress, dry weight of leaves, stems, roots, crown and total of oil palm seedlings reached the highest level at 0.71-0.83 g B/seedling.

Increasing doses of B >0.83 g/seedling was always followed by a decrease in leaf stomatal density of oil palm seedlings after drought stress treatment (Table 6). However, the increase in stomatal opening width had not been able to trigger an increase in stomatal conductance and transpiration rate. Table 8 also shows that any increase in Si application up to 4.61 g/seedling was always followed by a decrease in leaf stomatal density of oil palm seedlings after drought stress treatment. However, decreases in leaf stomatal density of oil palm seedlings were insufficient to decrease stomatal conductance and transpiration rate. Transpiration rate of the plants had a close relationship with stomatal conductance. Oil palm seedlings at various dose of B and Si have the same rate of transpiration because of all treatments tested had similar stomatal conductance (Table 9 and 10).

There was no regression relationship between application dose of B with the rate of photosynthesis but had a quadratic regression relationship with photosynthetic activity per plant (Table 11). Photosynthetic activity per plant reached a maximum at 0.71-0.83 g of B/seedling. Increasing doses of B >0.70 g/seedling lowered dry weight of leaves, stems, roots and total of oil palm seedlings, because of the availability of B in the soil beyond the requirement of oil palm seedlings. In the period following exposure to drought stress, dry weight of leaves, stems, roots, crown and total of oil palm seedlings reached the highest level at 0.71-0.83 g B/seedling.
There was no regression relationship between applications doses of Si with dry weight of leaves, stems, roots, shoot, total and root/shoot of oil palm seedlings, in the period of before and after exposed to drought stress (Table 14). Dry weight of plant parts had similar value among the varying doses of Si up to 4.61 g/seedling. However, the results of this study could not determine how the relationship between the doses of Si with all plant parts dry weight at doses >4.61 g/seedling.

Application dose of B had a quadratic regression relationship with plant height and stem diameter of oil palm seedlings, before and after exposure to drought stress (Table 15). In the period before the drought stress, plant height and stem diameter of oil palm seedlings reached a maximum at 0.82 and 0.50 g of B/seedlings, respectively. The application of B >0.82 g/seedling delayed the increase of trunk height while trunk diameters of oil palm seedlings begin to decline at dose of B >0.50 g/seedling. While in the period after drought stress, trunk height and diameter of oil palm seedlings reached a maximum at a dose of 0.64 and 0.73 g of B/seedling, respectively. The increase of trunk height of oil palm seedlings inhibited at application doses of B >0.64 g/seedling, whereas the increase in trunk diameter started to inhibit at 0.73 g of B/seedling.

Application dose of Si did not have a regression relationship with trunk height and diameter of oil palm seedlings, before and after the oil palm seedlings exposed to drought stress (Table 16). Trunk height and diameter of oil palm seedlings were similar among the varying dose of Si,
up to 4.61 g of Si/seedling. However, at the application dose of Si >4.61 g/seedling, the pattern of the relationship between the dose of Si with trunk height and diameter of oil palm seedlings could not be determined.

**DISCUSSION**

Application dose of B up to 1.75 g/seedling did not have a regression relationship with NRA, in the period of before and after drought stress (Table 1). Nonetheless, NRA in the leaf tissue of oil palm seedlings after drought stress period was significantly higher when compared with the period before the drought stress. The increase of NRA gave positive effects to maintain the activity of the metabolism of carbohydrates and protein during drought stress (Bellaloui, 2011; Garkar et al., 2011; Ananthi and Vijayaraghavan, 2012; Sepehr et al., 2012; Chaukiyal and Bhatia, 2014). Carbohydrates and proteins were substrates in the synthesis of various amino acids which act as antioxidants and osmoregulator to reduce osmotic potential of cells and protect cells from a variety of free radical compounds.

The increase of NRA in the period of after drought stress did not affect the chlorophyll content. Levels of chlorophyll a, b, total and also leaf greenness decreased significantly after a period of drought stress. The rise of NRA in the period after drought stress had not been able to inhibit the rate of chlorophyll degradation. In the period after drought stress, the ratio of chlorophyll a/b tends to increase. The increase in the ratio of chlorophyll a/b associated with degradation rate of chlorophyll a and b, where the degradation rate of chlorophyll a much slower than the one of chlorophyll b. Chlorophyll b damaged more severely due to direct interaction with high energy of sunlight during the drought period (Legros et al., 2009; Cha-um et al., 2010; Biglary et al., 2011; Cao et al., 2011; Dastan et al., 2011; Sun et al., 2011; Chutia and Borah, 2012; Khayatnezzhad and Gholamin, 2012; Rahimi et al., 2012; Zlatev and Lindon, 2012; Ashraf and Harris, 2013; Bharwana et al., 2013; Gomes et al., 2013). Oil palm seedlings allegedly degrade leaf chlorophyll and amino acids resulted from the degradation of the chlorophyll used as the base material to synthesize some important osmoregulator in osmotic adjustment (Legros et al., 2009; Cha-um et al., 2010; Cao et al., 2011; Sun et al., 2011; Chutia and Borah, 2012; Khayatnezzhad and Gholamin, 2012; Zlatev and Lindon, 2012; Ashraf and Harris, 2013).

Application dose of B also did not affect stomatal density and length of both upper and lower surfaces of leaves, except stomatal opening width of leaf below surface which tend to increase with increasing doses of B (Table 5 and 6). Drought stress treatment until the plant reached the permanent wilting point lead stomatal density, length and width were equal among the varying dose of B because the water contents were similar and all oil palm seedlings were used in the study had reached the permanent wilting point. The density and width of stomatal opening and stomatal conductance affected the rate of transpiration. The density and width of stomatal opening were not affected by the dose of B application, so that stomatal conductance and transpiration rate were relatively equal at all dose of B (Table 9). Stomatal conductance was positively correlated with transpiration rate (r = 0.43), so the situation in stomatal conductance associated with B application dose was similar to the transpiration rate. Stomatal conductance and transpiration rate of the plants after period of drought stress were smaller when compared to the normal condition (Al-Rawahy et al., 2007; Han et al., 2008; Ahmed et al., 2009; Hajiboland and Farhanghi, 2010; Hajiboland et al., 2012).

The rate of transpiration related to the diffusion of CO₂ into the leaf mesophyll. At the high level of transpiration rate, the diffusion of CO₂ into the leaf mesophyll was higher and vice versa. Drought stress inhibited the rate of transpiration so that the diffusion of CO₂ into the leaf mesophyll decreased (Ismail et al., 2004; Singh et al., 2006; Han et al., 2008; Ahmed et al., 2009; Hajiboland and Farhanghi, 2010; Hajiboland et al., 2012). The decline in the rate of transpiration also stimulated the activity of free radicals. Free radicals negatively impact the plant because it could damage cells and remodelled the organic compounds in the cell such as the chlorophyll.

Chlorophyll, water and CO₂ were the primary substrate of photosynthetic activity. The chlorophyll and transpiration rate was not affected by the dose of B application when plants were exposed to drought stress so that the rate of photosynthesis was equal among the varying dose of B (Table 11). However, the dose of B affected the photosynthetic activity per plant (Table 11), because the photosynthetic activity per plant was also determined by the total leaf area of plants. Photosynthetic activity per plant increased up to 0.33 g of B/seedling. Assimilates were produced by the activity of photosynthesis used by plants for cell maintenance and growth. The assimilate production of the plant that exposed to drought stress decreased due to the decrease of photosynthetic activity and metabolism by a decrease in leaf chlorophyll content (Choi and Latigui, 2008; Legros et al., 2009; Cha-um et al., 2010; Cao et al., 2011; Sun et al., 2011; Zlatev and Lindon, 2012; Ashraf and Harris, 2013; Fahramand et al., 2014; Zain et al., 2014). The decline of assimilate production significantly inhibited the growth of plants (Al-Amin et al., 2011).

Assimilate production from photosynthesis process was associated with dry matter produced. The dry material produced was used for plant growth which was indicated by changes in the physical volume in the form of trunk height and diameter (Al-Amin et al., 2011). Trunk height and diameter of oil palm seedlings after drought stress could be improved through the application of 0.64-0.73 g of B/seedling (Table 15). The application dose of B greater than 0.64-0.73 g/seedling tended to tamper the development of trunk height and diameter of oil palm seedlings (Table 15), associated with the toxicity effect of B, because at the toxicity level B actually interfered the physiological and metabolic activity of the plant cell.
Performance of plant morphology affected the dry weight produced, especially trunk height and diameter. Plants with larger physical volume had a greater dry weight as well. Since the trunk height and diameter were influenced by the dose of B, plant dry weight was also influenced by the presence of these elements (Table 13). Plant dry weight increased up to 0.83 g of B/seedling. There were positive correlation between trunk height and diameter with total plant dry weight (r = 0.67 and r = 0.62, respectively), that was why an increase in trunk height and diameter of oil seedlings was always followed by an increase in total plant dry weight (Al-Amin et al., 2011).

The application dose of Si to the oil palm seedlings did not have positive effects to the NRA, with an indication of a uniform NRA values among varying doses of Si. Nitrate reductase activity after drought stress period was much higher than before drought stress period. Nitrate reductase activity was not affected by the dose of Si applications, it was characterized by the absence of regression relationships between them (Table 2). The increase of NRA was indispensable for being able to maintain the activity of the metabolism of carbohydrates and proteins at high levels during periods of drought stress (Bellaloui, 2011; Garkar et al., 2011; Ananthi and Vijayaraghavan, 2012; Sepehr et al., 2012; Chaukiyal and Bhatia, 2014). Carbohydrates and proteins were substrates required in the synthesis of various amino acids. Amino acids were the basic material in the synthesis of organic compounds, employed as osmoregulator and antioxidant.

The increase of NRA in the leaf tissue of oil palm seedlings after period of drought stress did not give a positive effect on leaf chlorophyll content. Levels of chlorophyll a, b, total and also leaf greenness declined after a period of drought stress. Allegedly, an amino acid that was synthesized by the tissue, with the help of NRA, more focused to synthesize secondary metabolites such as phenols, proline and antioxidant because the compounds were needed to protect the cells from damage during drought stress period (Chakraborty and Pradhan, 2012; Abbas et al., 2014; Beig et al., 2014). The decrease in chlorophyll content during drought stress period was caused by high sunlight energy capture and low water content so that the chloroplast became vulnerable to damage (Biglary et al., 2011; Dastan et al., 2011; Rahimi et al., 2012; Bharwana et al., 2013).

The application dose of Si did not affect the density, length and width of stomatal opening (Table 7 and 8). The density and width of stomatal opening decreased after drought stress treatment and similar among varying doses of Si. The decrease in stomatal density and width of stomatal opening in the period of drought stress resulted in decreased of stomatal conductance. Stomatal conductance was not affected by the dose of Si application so that its value was equal among the varying dose of Si (Table 10). It was because the width of stomatal opening was large at all doses of Si application. There was a positive correlation between the width of stomatal opening and conductance (r = 0.46). The smaller of stomatal opening, lower of stomatal conductance. While stomatal conductance was positively correlated with transpiration rate (r = 0.43), a decrease in stomatal conductance was followed by a decrease in the rate of transpiration (Gong et al., 2005; Hou et al., 2006; Sacala, 2009; Putra, 2010; Putra et al., 2010a, b, 2012; Tale Ahmad and Haddad, 2011; Gong, 2011; Giuliani et al., 2013; Bouranis et al., 2014).

The rate of photosynthesis and photosynthetic activity per plant after drought stress period decreased when compared to the period of before drought stress. The application dose of Si did not affect the rate of photosynthesis and photosynthetic activity per plant (Table 12). Variables that affected the rate of photosynthesis and photosynthetic activity per plants were chlorophyll content per leaf (r = 0.47) and transpiration rate (r = 0.45) (Legros et al., 2009; Cha-um et al., 2010; Hajiboland and Farhanghi, 2010; Putra, 2010; Putra et al., 2010a, b, 2012; Cao et al., 2011; Sun et al., 2011; Upadhyay and Patra, 2011; Hajiboland et al., 2012; Zlatev and Lindon, 2012; Ashraf and Harris, 2013; Demiray and Dereboylu, 2013; Gupta and Solanki, 2013; Ancu et al., 2014; Fahramand et al., 2014; Zain et al., 2014). It has been described in the previous paragraph that the chlorophyll content of leaves and transpiration rate was not affected by the dose of Si application, therefore it was natural that the rate of photosynthesis and photosynthetic activity per plant was also not affected by the dose of Si application.

Dry matter was the result of photosynthetic activity. The decline in the rate of photosynthesis and photosynthetic activity per plant after drought stress period was followed by decrease in plant dry weight, both the shoot and root (Silva et al., 2013). The application doses of Si up to 4.61 g/seedling had not been able to provide a significant effect on plant dry weight (Table 14). These conditions were directly related to the situation prevailing at the rate of photosynthesis and photosynthetic activity per plant.

**CONCLUSION**

Applications of B and Si have the ability to induce physiological resistance of oil palm seedlings to drought stress. The mechanism of B to induce physiological resistance of oil palm seedlings to drought stress through the increase of leaf greenness, width of stomatal aperture and photosynthetic activity per plant. The optimal dose of B to induce physiological resistance of oil palm seedlings to drought stress ranged from 0.33-0.57 g/seedlings. The mechanism of Si to induce physiological resistance of oil palm seedlings to drought stress through the increase of leaf greenness and decrease the stomatal density of lower leaf surface. The optimal dose of Si to induce physiological resistance of oil palm seedlings to drought stress was 2.22 g/seedling.

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