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The Effect of Calcium on Photosynthetic Rate due to ABA and Proline Behaviour of Oil Palm (*Elaeis guineensis* Jacq.) Seedlings under Drought Conditions

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Abstract

Planting drought-resistance plants in terms of agronomy, such as induction of plant tolerance using calcium is assumed to be able to solve the climate anomaly problem. Calcium is known as an element that plays an essential role in determining the response of plant resistance to drought through biochemical activity. This study aimed to determine the role of calcium in changing photosynthesis activity in order to increase the resistance to drought stress. The treatment was arranged in factorial of 3 x 4 in a split plot Randomized Complete Block Design replicated three times. The first factor was the dose of calcium application consisted of 0 (control/without calcium), 0.04, 0.08 and 0.12 g. The second factor was the intensity of drought stress, which referred to the Fraction of Transpirable Soil Water method consisted of 1 (control/field capacity), 0.35 (moderate drought) and 0.15 (severe drought). The measurement data of stomatal aperture, Abscisic Acid (ABA) content, chlorophyll content, carotenoid content, proline content, nitrate reductase activity and photosynthesis rate that fulfill the assumption of homogeneity and normality were analyzed using variance at 95% accuracy and continued using DMRT. Moreover, regression analysis were determined of relationship between the treatment and parameters. The results revealed that drought resulted in a decline in leaf water potential and stomatal aperture. The effects of calcium on chlorophyll and carotenoid under drought stress could not be explained in this study. However, the application of calcium has a significant effect on decreased ABA, increased proline and nitrate reductase activity resulting in an increase in the photosynthetic rate of oil palm seeds in drought stress.

Keywords: ABA, carotenoid, chlorophyll, NRA, proline

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INTRODUCTION

Drought is the result of global climate change that harms plant growth and development, including oil palm. It was reported that the effects of drought in Sumatera, Indonesia had resulted in a 60% decrease in palm oil productivity (Darlan et al., 2016). There was 10% reduction throughout the year in production at every 100 mm of rainwater deficit (Carr, 2011). Plants response to drought stress through some biochemical, physiological and growth changes (Suguiyama et

al., 2016). Some of biochemical responses are closely related to photosynthetic activity including the decrease in leaf water potential, osmotic adjustment, osmotic protectant accumulation such as sugar alcohols, amino acids and organic acids (Bivi et al., 2016), the decreasing in carbon assimilation for photosynthesis due stomatal to closure, decreasing chloroplasts (Parry et al., 2005; Shekari et al., 2015) and photosystem II activity (Shekari et al., 2015). The tolerant cultivar that

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have better resistance to drought still showed a marked decrease in production by more than 50 percent due to the decline in photosynthesis efficiency (Jazayeri et al., 2015). However, drought considerably hampered the process of photosynthesis in most plants by altering the ultrastructure of the organelles and concentration of various pigments and metabolites including enzymes involved in this process as well as stomatal regulations (Ashraf and Harris, 2013).

Cultivation treatments were applied to overcome the problems by inducing calcium to strengthen plant resistance to the drought. Moreover, calcium has been well known to have an essential role in overcome abiotic stress, one of which is drought. It is an essential secondary messenger in the signal transduction pathway for various resistance responses. It was reported that Ca^{2+} ions were involved in a variety of basic regulation and process such as cytoplasmic flow, thigmotropism, gravitropism (Song et al., 2008), cell division, cell elongation, cell development, cell polarity, photomorphogenesis, plant defense and stress response (Reddy, 2001). It is involved in Abscisic Acid (ABA) transduction in guard cells, regulates the rate of transpiration, inhibits synthesis of reactive oxygen species (ROS), reduces malondialdehyde (MDA), increases antioxidant enzyme activity, increases enzyme catalase and proline content, protects plasma membrane structure, increases Rubisco activity, maintains photosynthetic efficiency, maintains nitrogen assimilation, regulates hormone metabolism and other essential chemicals as well. In addition to secondary messenger in conveying drought signals by regulating various physiological responses (Li et al., 2003; Shao et al., 2008; Song et al., 2008; Naeem et al., 2017).

Previous studies examined the induction of plant resistance using Silica and Boron. The application of Silica to oil palm seedlings can induce physiological resistance of oil palm seeds through increasing leaf greenery and decreasing the density of abaxial stomata (Dewi et al., 2014; Putra et al., 2015; Wirawan et al., 2016) while boron can increase green leaf index, stomatal aperture and photosynthetic activity per plant (Solihatun et al., 2014; Putra et al., 2016). However, the responses of plants to silica addition were more complex and it would risk producing severe toxicity if it had been applied on a large scale. On the other hand, the level of boron's sufficiency for plants have a narrow range so that it will be deficient in fewer quantities, but if it has the excessive quantities, it will be poison. Calcium is more likely to be carried out and has the potential to be successfully which applied on a broader scale in seedlings and higher plants since the plant's need for calcium that relatively high, so it is less possible to experience an overdose. Furthermore, the addition of calcium is useful for increasing the number of cations and improving soil fertility. It was improved the land optimization and supporting sustainable agriculture. This research aims to find out the role of calcium in the process of changing the photosynthesis activity of oil palm, especially in order to increase the resistance to drought stress.

MATERIALS AND METHOD

The experiment was conducted in August 2017 May 2018 in oil palm nursery, Bendosari, Village, Prambanan Sub-district, Madurejo Sleman District, Special Region of Yogyakarta. The observation of biochemical parameters was conducted in May - July 2018 at Plant Production Management Laboratory Faculty of Agriculture, Gadjah Mada University, Yogyakarta. The materials consist of Avros cultivar seeds that become a cultivar that was considered intolerant to drought from the Palm Oil Research Center, Medan. Planting media used was alfisols soil which had low fertility, calcium fertilizer (CaSO₄) pure analysis (PA) and NPK fertilizer for maintaining the seedlings.

Experiments were arranged according to the two-factorial randomized complete block design (RCBD). The first factor was drought stress consisted of FTSW (Fraction of Transpirable Soil Water) 1 (control/field capacity), FTSW 0.35 (moderate drought) and FTSW 0.15 (severe drought). The second factor was the application of calcium $(CaSO_4)$ consisted of 0 (no calcium/control), 0.04 gram; 0.08 gram and 0.12 gram. Each treatment combination consisted of nine plants that had been repeated three times with blocks as replications.

Seedlings maintained for four months at prenursery included irrigation, weed control and fertilization. The fertilizer given was NPK (15:15:15) with a dose of 3 gram per polybag applied every two weeks. Four months after transplanting (m.a.t) the seeds were transplanted and maintained during acclimatization in the main nursery for two months. The fertilizer given at the main nursery stage was NPK (15:15:15) with a dose of 5 gram per polybag. The calcium was given once in a ring placement starting when the seeds were 5 m.a.t. The calcium used was $CaSO_4$ (PA). Drought experiments were carried out by measuring the content of soil water that could be transpired (FTSW = fraction of transpirable soil water) referred to the method proposed by Ray

The weight of FTSW 1.00	=	1.00 (WFC - WPWP) + WPWP
The weight of FTSW 0.35	=	0.35 (WFC - WPWP) + WPWP
The weight of FTSW 0.15	=	0.15 (WFC - WPWP) + WPWP
WPWP	=	Weight of permanent wilting point
WFC	=	Weight of field capacity

Sinclair (1998).

The stress level of each plant was expressed as a function of soil water content. The rate of groundwater transpiration in each polybag on a particular day was calculated by the following equation:

and Sinclair (1998). The polybag was filled with

water, then weighed as field capacity (FC) when

the water was not dripping. The polybag was left

until the weight did not back decrease (stable)

then weighed as a permanent wilting point (PWP).

The weight of each level target of FTSW was

determined by the equation according to Ray and

$$FTSW = \frac{\text{weight of polybag on day } 1 - \text{weight of final polybag}}{\text{The weight of the initial polybag} - \text{the weight of the final polybag}}$$

Drought treatment was started at the age of eight months. The experiment was carried out for three weeks, two months after the application of calcium. That was the time when the seeds had been growing for six months then acclimatized. Environmental characteristics such as air temperature, relative air humidity and light intensity were measured every day at 09.00 am, 12.00 pm and 3.00 pm.

The variables included leaf water potential, stomatal aperture, proline content, nitrate reductase activity, chlorophyll a content, chlorophyll b content, total chlorophyll content, carotenoids content and photosynthesis rates. For all these measurements, the material was a leaf organ, especially one that was located on the top of the canopy and had perfectly expanded. The leaf water potential (Ψ w) was measured using a pressure chamber instrument model 1000, while stomatal aperture measurements using the method developed by Hilu and Randall (1984). Analysis of nitrate reductase activity was performed using a method developed by Hartiko (1987), while proline content in leaf tissue was extracted and analyzed according to the method proposed by Bates et al. (1973). Chlorophyll and carotenoid contents were measured according to method Lichtenthaler and Buschmann (2001). Net Photosynthetic Rate (Pn) was calculated by Photosynthetic Analyzer type LI Cor LI 6400. The data were then analyzed using variance (ANOVA)

 $\alpha = 5\%$ significantly and regression to determine the relationship between the treatment and parameters. When the F test showed a significant effect, it will be continued with DMRT (Duncan's Multiple Range Test) $\alpha = 5\%$.

RESULTS AND DISCUSSION

Based on the preliminary experiment, the value of soil water content in the field capacity (FC) was equivalent to soil water content (SWC) by 46.9% and the wilting point (PWP) was equivalent to 25%. In line with the results of measurements in the field, a permanent wilting point was obtained at 11.055 kg, the target weight of FTSW 0, 0.35 and 0.15 were determined for each polybag. Based on the gravimetric method, the measurement of soil moisture content of FTSW 0.35 was equivalent to SWC by 32.66%. Moreover, FTSW 0.15 was equivalent to SWC 28.28%. Polybag weighing was done every day at 6.00 am, with an average daily temperature of 29 °C – 42 °C.

Assessment of decreasing in leaf water potential could be an indicator of the impact of drought intensity with a reduction value of 0.1 MPa if the plants had a mild stress, 1.2 - 1.5 MPa if the plants had a moderate stress and >1.5 MPa if the plants had a severe drought (Ai and Banyo, 2011). Regression analysis showed a linear relationship between moisture content and leaf water potential, which decreased moisture content followed by a decrease in leaf water potential. The average leaf water potential in the field capacity condition was -0.49 MPa down to -1.55 MPa at moderate drought and decreased again to -2.88 MPa in severe drought. The decrease of leaf water potential in this research indicated that the status of water in the leaf when soil water content by 32.7% had moderate stress and it had severe stress when the soil water content was 28.3%. The potential decline in oil palm leaves was caused by the decline of potential groundwater (Jazayeri et al., 2015; Silva et al., 2016).



Figure 1. Regression between moisture content and leaf water potential after drought stress



Figure 2. The stomatal aperture (µm) in some soil water content under drought stress



Figure 3. Regression between ABA content and soil water content

Drought stress had been shown to reduce stomatal aperture compared to controls. There were no significant differences between the effects of moderate stress and severe stress in reducing the stomatal aperture. The data showed that the drought intensity level did not limited by the decline in stomatal aperture. The decline in stomatal aperture is the first line of defense against dehydration (Hopper et al., 2014). The regulation of stomatal aperture was based on the level of the water deficit and may close partially. allowing carbon fixation during drought conditions with improved water use efficiency (Benesova et al., 2012). The decrement of soil moisture is the first signal received by the root that responded by the plant through stomata closure to reduce transpiration. This is the performance of ABA whose concentration increases from the root to the xylem sap then to the leaf organ. ABA content in the plant under drought is generally higher than in field capacity (Fathi and Tari, 2016). In this experiment, ABA content increased linearly with reduced soil water content. The increase in ABA concentration is a plant response to the drought signal which mediates stomatal closure to reduce transpiration. The increase in ABA concentration under drought is in line with the statement (Zhang and Jr, 2001; Fathi and Tari, 2016; Zhu, 2017) that ABA is an important stress marker hormone that accumulates and causes the stomatal closure when the plant experiences osmotic stress such as drought and high salinity.

 Table 1. The effect of calcium on ABA content at calcium application levels (ppm)

Calcium (g)	Mean
0.00	3.27 ab
0.04	3.71 a
0.08	2.51 bc
0.12	2.18 c
CV 25.10%	

Note: Different letters in each column show a significant effect at $p \le 0.05$ by Duncan's New Multiple Range Test (DMRT)

Application at the lowest dose of calcium produced the highest ABA. On the other way, the ABA measurements showed that the application of calcium at a dose 0.12 g significantly lower than control but no different with 0.08 g calcium. It was commonly known that plants responded to the decrease in leaf water potential through stomata closure regulated by ABA. Allegedly, the high calcium content in seeds with the application of 0.08 and 0.12 g could increase plants sensitivity to the increase in ABA concentration, particularly while experiencing moderate drought. A review

of ABA signaling in stress response and seed development conducted by Nakashima and Shinozaki (2013) mention that Ca²⁺ signaling might have important roles for ABA signaling. Furthermore, Asano et al. (2012) stated that increasing the calcium sensor in rice seedlings causes an increase in plant sensitivity to ABA. ABA could induce the expression genes that were controlled by stressors, but this gene could also be expressed by the presence of Ca²⁺ and Ca²⁺-carrier even though there were no stress signals that induces ABA. The statement showed that the expression of stress marker genes was mediated by the presence of Ca^{2+} (Reddy, 2001). The result explained that the plants with the highest calcium were more responsive to stress signals without causing more ABA accumulation. As a result, the stomata not fully close and could maintain its conductivity and CO₂ diffusion as well.

The measurement of chlorophyll content can be used to determine the efficiency and function of photosynthesis in plants that experience drought stress (Cha-um et al., 2010; Cha-um et al., 2013). The results revealed the influence of the interaction between calcium application and drought stress on chlorophyll a content. Without calcium the effects of drought on chlorophyll a content were not significantly different among SWC levels, yet tended to be higher when experiencing moderate drought. Whereas in field capacity, the application of calcium could increase the chlorophyll a content as doses increase. The chlorophyll a content with 0.12 g calcium was significantly higher than control. In moderate drought, the application of 0.08 and 0.12 g of calcium actually resulted in lower chlorophyll a than the control and a dose of 0.04 g could provide the highest one. While seedlings exposed to severe drought, there were no significant differences between all calcium doses compared to control, but a dose of 0.04 g tended to produce the lowest chlorophyll a content. In seedlings with 0.04 g of calcium, the chlorophyll a content was higher in moderate drought and not different from field capacity. The data showed that calcium was proven to be able to prevent chlorophyll a degradation even while experiencing mild stress when it was given in small amount (0.04 g). In contrast, 0.04 g sharply decreased chlorophyll a even compared to the field capacity when the seeds experience severe drought.

Calcium (g) —		Mean		
	46.9	32.7	28.3	
Chlorophyll a				
0.00	3,820.71 bcd	4,516.53 abc	3,712.29 bcd	4,016.51
0.04	4,270.91 abc	4,767.03 a	3,234.49 d	4,090.81
0.08	4,572.21 ab	3,647.49 cd	4,238.44 abc	4,152.71
0.12	4,706.97 a	3,362.11 d	3,727.03 bcd	3,932.04
Mean	4,342.70	4,073.29	3,728.06	(+)
CV FTSW		7.20%		
CV Calcium		11.93%		
Chlorophyll b				
0.00	3,952.39 bcde	5,200.37 abc	3,703.15 cde	4,285.30
0.04	4,808.89 abcd	5,619.27 a	2,633.34 e	4,353.83
0.08	5,209.07 abc	3,496.25 de	4,740.53 abcd	4,481.95
0.12	5,509.95 ab	2,891.57 e	3,646.59 cde	4,016.04
Mean	4,870.07	4,301.87	3,680.90	(+)
CV FTSW		16.05%		
CV Calcium		19.91%		
Total chlorophyll				
0.00	7,770.65 bcd	9,713.77 ab	7,413.12 bcd	8,299.18
0.04	9,076.89 abc	10,382.93 a	5,866.09 d	8,441.97
0.08	9,778.13 ab	7,141.53 cd	8,976.09 abc	8,631.92
0.12	10,213.61 a	6,251.80 d	7,371.33 bcd	7,945.58
Mean	9,209.82	8,372.51	7,406.66	(+)
CV FTSW		11.75%		
CV Calcium		15.97%		

Table 2. The effects of interaction between drought and calcium levels on chlorophyll a, chlorophyll b and total chlorophyll content (mg. L⁻¹)

Note: Different letters in each column and row show a significant effect at p ≤ 0.05 by Duncan's New Multiple Range Test (DMRT). (+) indicates the treatment interaction

The similar results also occurred in chlorophyll b and total chlorophyll content, where in the moderate drought conditions, calcium addition tended to reduce the chlorophyll b and total chlorophyll content. However, in a severe drought there were no differences between the application of calcium and controls. The result showed that although drought has been shown to reduce the chlorophyll a, chlorophyll b and total chlorophyll content, the effects of calcium application variably depends on the drought level.

Carotenoids are present in the chloroplasts and are bound in photosystem I and photosystem II complexes, so they have the main task in the first protection from oxidative damage (Chakraborty and Pradhan, 2012) and one of the important nonenzymatic antioxidants (Jaleel et al., 2009; Silva et al., 2016). Based on the analysis of variance, there was an interaction effect of calcium application treatment with drought stress on carotenoid content. Generally, the decrease in SWC had no effects on the carotenoid content of seeds without calcium, although it tended to be higher when experiencing drought. Carotenoid as the non-enzymatic antioxidants might represent a strategy of oil palm plants to cope with drought induced oxidative stress (Silva et al., 2016). On the other hand, in field capacity, there was no significant difference between the calcium dosage application for carotenoid content, although it tended to be higher with applied of calcium. In moderate drought application of 0.04 g calcium

produced a significantly lower carotenoid content than the control, although it was not significantly different with other doses. Meanwhile, in the case of severe drought stress, the application of 0.04 g of calcium gave significantly higher carotenoid content than 0.08 g calcium but no significant compared with control. However, the plant response showed that the application of calcium did not increase the carotenoids during drought.

Calcium (g)		SWC (%)		
	46.9	32.7	28.3	
0.00	465.02 bc	535.86 ab	538.21 ab	513.03
0.04	550.64 ab	397.93 c	635.54 a	528.04
0.08	545.15 ab	483.14 bc	434.97 bc	487.75
0.12	556.49 ab	456.05 bc	565.99 ab	526.18
Mean	529.33	468.25	543.68	(+)
CV FTSW		13.	38%	
CV Calcium		14.	17%	

Table 3. The effect of interaction between drought and calcium levels on carotenoid content (mg. L⁻¹)

Note: Different letters in each column and row show a significant effect at $p \le 0.05$ by Duncan's New Multiple Range Test (DMRT). (+) indicates the treatment interaction

Table 4. The effect of drought and calcium levels on NRA (umol NO₂⁻ g^{-1} hour⁻¹)

OII NKA (µIIIOI NO2 g IIOuI)			
Treatment	Mean		
SWC (%)			
46.9	1,356.73 b		
32.7	2,531.54 a		
28.3	2,199.24 a		
CV SWC	33.99%		
Calcium (g)			
0.00	1,558.59 b		
0.04	2,936.23 a		
0.08	1,863.26 b		
0.12	1,758.61 b		
CV Calcium	39.16%		

Note: Different letters in each column show a significant effect at $p \le 0.05$ by Duncan's New Multiple Range Test (DMRT)

The major enzyme's activity such as nitrate reductase involved in the nutrient metabolism can also be significantly reduced under high temperature stress (Fahad et al., 2017). The condition is related to the decline in stomatal aperture and the concentration of CO_2 in the intercellular space which is known to reduce the activity of various enzymes including nitrate reductase (Chaves et al., 2008). The results showed that there was no interaction between the application of calcium and drought stress on the NRA. However, there was a single effect of drought stress and calcium application on the

NRA. NRA in moderate and severe drought stress was significantly higher than the field capacity. The application dose of 0.04 g calcium could also significantly increase NRA compared to controls and other doses. This confirmed that during drought, the plant carried out a mechanism for increasing the activity of nitrate reductase as a response to abiotic stress adaptation, as shown by (Azcon and Tobar, 1998; Sepehr et al., 2012) experiment. The NRA characteristics were related to the increase in proline in this experiment. (Sharma and Dietz, 2006) reported that the increase in proline would reduce protein aggregate and denaturation due to temperature changes. In an in vitro experiment, the proline protected the activity of nitrate reductase under osmotic, metal and H₂O₂ stress.

The increase of proline content is the most sensitive plant response to reduced leaf water potential and increased osmotic pressure (Carvalho, 2008; Lokhande and Suprasanna, 2012; Liang et al., 2013). Based on the result, there were no significant differences in the effect of calcium on proline content in field capacity. Conversely, in the moderate drought there were no significantly different effects between 0.04, 0.08 and 0.12 g calcium on proline content, but significantly higher than control. At severe drought, the application 0.04 and 0.08 g calcium was not significantly different from the control, but was significantly higher than 0.12 g calcium which became the lowest proline content

compared to all treatments. Without the presence of calcium, the proline content decreases until moderate drought then sharply increases with reduced SWC. This pattern shows that plants are

less sensitive to drought signals even though they eventually increase after SWC levels gradually decline.

Table 5. The effect of inte	eraction between o	drought and cal	cium levels on prolir	e content (µmol g ⁻¹)
Calcium (g)		SWC (%)		Mean
	46.9	32.7	28.3	
0.00	50.30 b	25.60 c	112.73 a	62.88
0.04	43.32 bc	115.31 a	111.34 a	89.99
0.08	36.76 bc	118.09 a	119.38 a	91.41
0.12	37.04 bc	108.44 a	42.25 bc	62.58
Mean	41.85	91.86	96.43	(+)
CV FTSW		30.88%		
CV Calcium		11.20%		

Note: Different letters in each column and row show a significant effect at $p \le 0.05$ by Duncan's New Multiple Range Test (DMRT). (+) indicates the treatment interaction

According to Knight et al. (1998) that signal delivery requires sufficient calcium to induce the expression of genes involved in proline biosynthesis. Conversely, the addition of calcium proved to be able to induce an increase in proline since SWC levels began to decrease. This proves that calcium addition plays an important role in signal transduction which induces the expression of the *p5cs* gene. The low proline content in the application of 0.12 g calcium in severe drought indicated the presence of other mechanisms that arise when plants get more calcium. This is related to the decline in ABA content at that dose because proline accumulation requires ABA induction when the plant is exposed to water deficit (Carvalho, 2008; Liang et al., 2013).

Table 6. The effect of interaction between drought and calcium levels on photosynthesis rate (µmol $m^{-1} s^{-1}$

Calcium (g) —		Maan		
	46.9	32.7	28.3	Mean
0.00	357.33 a	157.33 e	181.67 de	232.11
0.04	256.00 bcd	250.67 bcde	251.67 bcde	252.78
0.08	244.00 bcde	217.67 cde	209.33 cde	223.67
0.12	160.67 e	320.67 ab	277.67 abc	253.00
Mean	254.50	236.58	230.08	(+)
CV FTSW	5.41%			
CV Calcium	22.45%			

Note: Different letters in each column and row show a significant effect at $p \le 0.05$ by Duncan's New Multiple Range Test (DMRT). (+) indicates the treatment interaction

Table 6 informs that there was an interaction between the application of calcium and drought the photosynthetic rate. stress on The photosynthetic rate of seeds without calcium application is decrease when SWC is decrease from moderate to severe. This is related to the reduction in CO₂ fixation when the stomatal aperture decreases in cells that experience osmotic

stress. While the increase of photosynthetic rate in seeds with a dose of 0.12 g calcium was present due to moderate and severe drought stress associated with a decline in ABA content. Calcium ions are reported to be involved in the opening of stomata when abscisic acid is not present (Brandt et al., 2015). The high level of Ca^{2+} in the guard cells was a signal to reduce

ABA. It made the stomata partially close and allowed the continuity of CO₂ diffution. It is also believed that the increase of photosynthetic rate involves the needs for dissolved sugar as osmolyticum and an ingredient in ATP metabolism in leaves. According to Pego et al. (2000) the decline in sugar content activates the expression of assimilates enrichment genes and increases photosynthetic capacity, whereas when the sugar excess and it will suppress gene expression for photosynthesis and photosynthetic rate. In field capacity, the application of calcium tended to reduce the photosynthetic rate, conversely happened in drought stress. This experiment showed that plants have a positive response to the application of calcium through an increase in photosynthesis rate in drought. In tobacco plants exposed to heat stress, the application of exogenous calcium chloride (CaCl₂) (20 mM) improved the process of photosynthesis concerning improving the stomatal conductance and the thermostability of oxygen-evolving complex, which might be due to less accumulation of reactive oxygen species (Tan et al., 2011).

Based on the description of each variable show that the application of calcium affects various biochemical processes that lead to the scheme of drought resistance in oil palm seedlings. This was characterized by a decline in ABA leaf content to prevent the stomata fully close so that CO₂ diffusion might be maintained. The availability of CO_2 in intracellular space is a limitation for the process of photosynthesis. The condition was supported by an increase in proline as an osmolytic, as well as an antioxidant that protects the enzyme from degradation, so the nitrate reductase activity was maintained. Although, the degradation of chlorophyll and carotenoids could not be prevented, it could be stopped at certain calcium levels. The photosynthesis rates continue to increase through an adequate supply of CO₂, besides driven by the plants which need to produce assimilates and various adjustments to cope with the drought stress.

CONCLUSIONS

This study revealed that calcium as an essential element can be used to reduce the effects of drought on plants by increasing the rate of photosynthesis to prevent the stomata from closing completely so that the diffusion of CO_2

continued and increased the NRA and proline accumulation to maintain cell turgor and prevented the accumulation of free radicals. The effect of calcium on chlorophyll and carotenoid under drought stress could not be explained in this study, so it is recommended for further investigation.

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