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Drought Stress Affecting Growth and Some Physiological Characters of Three Cocoa Clones at Seedling Phase

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Abstract

Drought stress can have substantial negative impacts on cocoa plant growth until affect the death of plant. The present study aimed to determine the effect of drought stress on the some physiological characters of cacao (*Theobroma cacao* L.) seedlings. The research was carried out at the Indonesian Coffee and Cocoa Research Institute, Jember, East Java, Indonesia. This research was conducted from January–December 2017. The experiment was designed by using completely randomized block design with two factors and with three replications. The first factor was clones, namely ICS 60, Sulawesi 1 and KW 641 clones. The second factor was interval of watering treatment, namely watering every 2 days (control/ adequate water condition), watering every 5 days (moderate stress), and watering every 8 days (severe stress). Result of this study concluded that drought stress reduced the growth of cocoa seedlings, mainly as a result of reducing photosynthetic activities in all stressed cocoa clone seedlings. Under drought stress, KW 641 and Sulawesi 1 had higher leaf area, chlorophyll a, chlorophyll b, total chlorophyll content, relative water content, and photosynthetic rate than ICS 60 clone.

Keywords: Growth, drought stress, photosynthetis, *Theobroma cacao* L.

INTRODUCTION

Cocoa (*Theobroma cacao*) is a most important plantation crop in the world. The bean produced are for the production of chocolates and confectionaries. However, cocoa production is highly sensitive with the changes of weather condition (Santosa *et al.*, 2018). Ideally, cocoa requires a minimum 1500-2000 mm of rainfall with less than 3 months of dry season (Baon, 1988). However, ENSO (El Nino Southern Oscillation) cause prolonged dry season in the past decade and lead to occur abiotic stress. Abiotic stress, especially drought stress that related to El Nino phenomenon, is noted that can decrease global cocoa production up to 15% (ICCO, 2010). In Sulawesi, it was found that ENSO-related drought caused a 62% loss of cocoa production (Keil *et al.*, 2008). Furthermore, Gateau-Rey *et al.* (2018) explained that drought stress can have substantial negative impacts on plant growth until the death of plant up to 15%.

The detrimental effects of drought stress on the growth and yield of cocoa are well documented (Gateau-Rey *et al.*, 2018; Keil *et al.*, 2008; Schroth *et al.*, 2016). It is therefore of great importance not only to know growth and yield of cocoa, but also to understand the process of physiological

level in this stress. Under drought stress, Medeiros *et al*. (2012) reported that the inflow of water reduces due low water availability in soil, which potentially affects the physiological processes dependent on turgor pressure because of low water status in plant. To know plant water status, relative water content (RWC) is important variable that commonly studied to assess plant physiological responses to water stress (Silva *et al.*, 2009; Marchese *et al.*, 2010; Silva *et al.*, 2010; Medeiros *et al.*, 2012). Moreover, this adverse condition will inhibit physiological process and then decrease the yield of cocoa.

Plant generally has mechanism to minimize water loss during stress condition. Stomatal closure is a strategy of cocoa plant for diminishing water loss through transpiration process by regulating stomatal conductance (Zakariyya *et al.*, 2016). Stomatal conductance can be defined as the ability of stomata that plays a role on CO_2 uptake and water loss through transpiration. That was determined by the degree of stomatal aperture and therefore the physical resistances to the movement of gases between the air and the interior of the leaf (Pietragalla & Pask, 2012). In other hand, stomatal closure also consequently reduces CO_2 uptake.

Drought stress also inhibit the activity of enzymes, such as nitrate reductase (Mandi *et al*., 2018). Then, it also causes the degradation of photosynthetic pigment, such as chlorophyll. The degradation in the concentration of photosynthetic pigments causes damage in photochemical activity. Chlorophyll, water and CO_2 are main components for photosynthesis process and then inhibit plant growth. Measuring physiological character, especially chlorophyll, photosynthesis, and gas exchange is an effective approach to understand effect of water stress on growth and production, because this character is highly correlated with yield of cocoa (Zakariyya *et al*., 2015; Anita-sari *et al*., 2015). In other case, physiological traits for drought tolerance were evaluated in a recent study to identify the potential of certain traits to be used as a way to accelerate breeding during early screening (Alban *et al.*, 2016). The present study aimed to determine the effect of drought stress on the growth and some physiological charater of cacao (*Theobroma cacao* L.) seedlings.

MATERIALS AND METHODS

The research was carried out at the glasshouse of Indonesian Coffee and Cocoa Research Institute (ICCRI), Jember, East Java, Indonesia. This research was conducted from January–December 2017. The relative humidity and average temperature during the experiment are measured during experiment period. The relative humidity ranged between 76 and 97% and temperature between 26.9 and 28.5^{O}C. Three cocoa scions, viz. ICS 60, Sulawesi 1, and KW 641 was grafted on to Scavina 6 halfsibs clone as a rootstock. Plant were grafted 5 months after sowing by using top grafting technique. Each polybag was fulfilled with 12 kg soil with a density of 1.1 g cm^{-3}.

The experiment was designed by using completely randomized block design with three replications. The first factor was clones, i.e. ICS 60, Sulawesi 1 and KW 641 clones. The second factor was interval of watering treatment, i.e., watering every 2 days (control/ no stress), watering every 5 days (moderate stress), and watering every 8 days (severe stress). Some variables of growth component that were measured include plant height, stem diameter, and leaf area. Plant height was measured by using ruler. Stem diameter was measured by using caliper. Leaf area meter was measured by using leaf area meter. Plant height, stem diameter, and leaf area was measured on 80 days after treatment. Photosynthesis rate, stomatal conduction,

transpiration rate, leaf temperature, and intercelullar CO_2 of cocoa seedlings was measured with a portable photosynthesis system analyzer Li-Cor 6400 (Licor Inc Lincoln Ne USA). For this measurment, the equipment was tagged on the youngest fully expanded leaf which are reported to be the most physiologically active.

Chlorophyll content was tested by using spectrophotometric method on 80 days after treatment. 0.1 gram of leaf was pulverized by using mortar and then extracted with 50 mL acetone 80%. The sample then was filtered by using filter paper in Erlenmeyer glass. The extract was put into cuvvet to measure the absorbance with spectrophotometer (varian cary model 50 conc) at 663 nm and 646 nm wavelength. As blank was used acetone 80%. Chlorophyll content was determined as follows : Chlorophyll a = 12.21 x A663–2.81 x A646 x 50; Chlorophyll b = 20.13 x A646–5.03 x A663 x 50; and total chlorophyll content = chlorophyll a + chlorophyll b.

The activity of nitrate reductation was analyzed by using spectrophotometric method. 0.2 gr leaf was cut slightly. Dark movie tube was prepared and added with 5 mL 0.1 M phospate buffer (pH 7). The cut leaf was put into tube that had been added with buffer phospate and waited for 24 hours. After 24 hours of immersion, it was replaced with new buffer solution and added 0.1 mL $NaNO_3$ 5 M as substrat, then incubated for 2 hours. In the same time, Dye reagent consisted of 0.2 mL of 1% sulphanilamide in 3 N HCl and 0.2 mL 0.02% N-Naphtylethylene diamine was prepared in a test tube. After incubation for 2 hours, 0.1 mL of filtrate was taken from a dark movie tube and put into the test tube containing the dye reagent, and then wait until it was a pink as a sign to reducing nitrate to nitrite by nitrate reductase enzyme. One test tube is not given the filtrate and used as a blank. After the change of color added 2.5 mL of distilled water, and moved in the cuvvette of spectrophotometer, and the observed absorbance at a wavelength of 540 nm. Correlation and regression analysis was conducted to determine the relationship among of variables. Data were analyzed using analysis of variance, if there is a significant difference then it is continued with Tukey test at 5% level.

RESULTS AND DISCUSSION

Cocoa growth was represented in Table 1. Watering interval treatment was affected in plant height, stem diameter, and leaf area variables. The increasing of watering interval from 2 days to 5 days significantly reduced only leaf area, but the increasing of watering interval from 2 days to 8 days reduced all growth parameters. The difference in plant height, stem diameter, and leaf area between regularly watering and severe drought stress (watering every 8 days) seedlings was about 10.17 %, 10.19 %, and 25.63 %, respectively. This was similar with the study of Chibuike & Daymond (2015) that reported there was a significant decrease, approximately more than 10 % of reduction, in plant height with the increasing of watering interval treatment. In addition, Lahive *et al.* (2018) also showed that leaf area and stem diameter declined up to 20% in response to water deficit. Therefore, it be concluded that drought stress was significantly induced the reduction of vegetative stage in cocoa.

The activity of some enzymes of plant metabolic pathway was regulated by water (Lisar *et al*., 2012). Nitrate reductase is one of the many enzymes that sensitive to water stress (Fresneau *et al*., 2007). This enzyme plays role in catalyzing nitrate to be nitrite. This enzyme is the regulator of nitrogen assimilation in cocoa plant (Armendariz *et al*., 1991). According to Table 2., the activity of nitrate reductase was affected by watering

interval treatment. Watering interval every 5 days (moderate drought stress) and every 8 days (severe drought stress) relatively decreased this enzyme activity by 46.98% and 61.57% than that watering every 2 days. The result proved that more severe drought stress level, the lower the activity of nitrate reductase. Several studies also showed that drought can decrease this enzyme activity (Ananthi & Vijayaraghavan, 2012; Chachar *et al.*, 2016; Mutjaba *et al.*, 2016; Mandi *et al.*, 2018). Under drought stress, Hernandez-Cruz *et al.* (2015) stated that a decrease in nitrate reductase activity was caused by the low nitrogen status in plant, especially nitrate compound. Santos *et al.* (2014) represented that soil water deficit significantly reduced leaf nitrogen content for all cocoa genotypes. Furthermore, Siswanti & Agustin (2014) stated that how drought stress can decrease this enzyme activity because water was useful to donate the proton and electron for the activity of nitrate reductase, spesifically, in each changes of nitrate to be nitrite process, water had to donate six electron for its process. The reduction of nitrate is mediated by nitrate reductase to generate nitrite. Nitrite is converted to be amonium through nitrate reductase. In other hand, nitrate reductase plays a role to synthesize nitrate oxide, which it is a molecule that is recognized as a signal transduction in plant.

Tabel 2 also demonstrated that watering interval treatment influenced on chlorophyll a content, chlorophyll b content, and total chlorophyll content. Watering every 8 days could decrease chlorophyll a content, chlorophyll b content, and total chlorophyll content until 42.89%, 63.39%, dan 53.57%, respectively, than regularly watering (every 2 days). In addition, chlorophyll a and total chlorophyll was also affected by clones. ICS 60 clone had lower chlorophyll a and total chlorophyll content than both KW 641 and Sulawesi 1 clones. More decrease in ICS 60 clone indicated that it is more sensitive to water deficit than KW 641 and Sulawesi 1 clones.

A decrease of chlorophyll content is a generally observed phenomenon under this stress. Prihastanti (2010) also reported the cocoa that grown on low soil water content have chlorophyll a and b are lower than those grown on adequate soil water content. Chlorophyll a is the most generally used the pigment of photosynthetic and it absorbs blue, red and violet wavelengths in the visible spectrum. It is also involved in oxygenic photosynthesis that oxygen is the main product of that process. In another side, chlorophyll b absorbs blue light and is used to extend the range of light wavelength. Chlorophyll a and b work simultaneously to absorb blue to red spectrum of light.

Tabel 1. Plant height (cm), stem diameter (mm), and leaf area (cm^2) of three cocoa clones at different watering interval treatment

Watering interval (days)	Plant height	Stem diameter	Leaf area
2 (normal)	74.02 a	11.97 a	2273.48 a
5 (moderate stress)	69.61 ab	11.43 a	1803.86 b
8 (severe stress)	66.49 b	10.75 b	1690.61 b
Clones			
ICS 60	68.99 p	11.30 p	1645.53 p
KW 641	70.39 p	11.23 p	2034.45 q
Sulawesi 1	70.74 p	11.62 p	2087.98 q
Interactions	(-)	(-)	(-)

Note: Values on the same column in each variable followed by the same letter were not different based on the Tukey test with α = 5%. (-): There was no interaction.

Tanaka & Tanaka (2005) has been demonstrated that chlorophyll b is synthesized from chlorophyll a through enzyme activity, and it was highly correlated with soil water availability. Lisar *et al.* (2012) stated that water stress inhibits the synthesis of chlorophyll through several stages namely (a) the synthesis of 5-Aminole-Vuliniuc Acid (ALA). (b) the condensation of this compound into porphobilinogen and primary tetrapyrrol, that is transformed into protochlorophyllide; (c) light-dependent conversion of protochlorophyllide into chlorophyllide; and (d) chlorophylls a and b synthesis along with their inclusion into developing pigment-protein complexes of the photosynthetic apparatus. In other case, under drought stress, chlorophyll pigment was also degradated by the existence of ROS due to oxidative stress (Rogers & Munne-Bosch, 2016).

Cacao leaf water content are used to be an indicator of plant water status. The relative water content also strongly correlated with water potential in plant tissue (Gholami *et al*., 2012). Table 3 informed that relative water content was affected by the interaction between clones and watering interval treatment. Under normal condition (watering every 2 days), there were no significant differences in all clones. Watering every 5 days significantly decreased relative water content in ICS 60 clones up to 13.73% than watering every 2 days. Meanwhile, the increasing of watering interval from 2 days to 8 days induced the reduction of relative water content in all clones. It indicated that in moderate drought stress level, ICS 60 clone had low plant water status than others.

In young cocoa that was grown under similar environmental conditions, leaf water potential were significantly reduced when watering was withheld for 10 days (Bae *et al*., 2008). Moreover, Almeida *et al*. (2016) reported that water deficit stress resulted in reduced leaf water potential and relative water content (RWC) with the former being a more sensitive indicator of plant water status. The results of Balasimha *et al*. (2013) trials indicated that the genotypes showing higher water potential can be considered as drought tolerant. The decreasing of RWC in leaf under drought stress may depend on the reduction of plant vigor. Blokina *et al*. (2003) stated that under drought stress, cell membrane subjects to changes such as penetrability and decrease in sustainability. Arjenaki *et al*. (2012) reported that microscopic observation of dehydrated cells, revealed damages including cleavage in the membrane and sedimentation of cytoplasm content.

Tabel 2. The activity of nitrate reductase, chlorophyll a, chlorophyll b, and total chlorophyll content of three cocoa clones at different watering interval treatments

Watering interval (days)	Nitrate reductase activity	Chlorophyll a content	Chlorophyll b content	Total chlorophyll content
2 (normal condition)	2.81 a	18.09 a	19.31 a	37.39 a
5 (moderate stress)	1.49 b	15.88 a	14.90 a	30.77 b
8 (severe stress)	1.08 b	10.33 b	7.04 b	17.36 b
Clones				
ICS 60	1.53 p	12.35 p	11.23 p	24.18 p
KW 641	1.94 p	16.39 q	15.30 p	31.59 q
Sulawesi 1	1.91 p	15.56 q	14.72 p	30.37 q
Interactions	(-)	(-)	(-)	(-)

Note: Values on the same column followed by the same letter were not different based on the Tukey test with α = 5%. (-): There was no interaction.

Tabel 3. Relative water content (%) of three cocoa clones at different watering interval treatments

Clones	Watering interval (days)		
	2	5	8
ICS 60	86.63 a	72.90 bc	60.83 d
KW 641	86.66 a	81.22 ab	74.18 bc
Sulawesi 1	86.31 a	79.45 ab	76.42 b
Interactions	-	(+)	-

Note : Values on the same column followed by the same letter were not different based on the Tukey test with α = 5%. (+): There was interaction, (-): There was no interaction.

Based on Table 4 there was no interaction between clone and watering interval treatment on stomatal conductance, transpiration rate, and intercelullar CO_2 concentration. Single factor of watering interval affected on that variables. Watering every 8 days could reduce stomatal conductance, transpiration rate and intercelullar CO_2 concentration. Spesifically, severe drought stress caused the reduction of stomatal conductance, transpiration rate, and intercelullar CO_2 concentration than that in normal condition. It described that the severe drought stress level would simultaneously reduce the activity of stomata and gas exhange. In other hand, single factor of clone affected on stomatal conductance and transpiration rate. Sulawesi 1 and KW 641 has lower both stomatal conductance and transpiration rate than ICS 60.

The various in stress severity was reffered to the response of stomatal and gas exchange between clones. This study were reported by Balasimha *et al.* (2013), who concluded that effective stomatal regulation is a key drought tolerance response of cacao that can result in decreased transpirational water loss. The ability of crops to maintain water potential values and turgor under water-limiting conditions is an important physiological adaptation towards periods of reduced water availability. Drought stress implicated to stomatal closure and then could decrease CO_2 diffusion from the atmosphere.

Table 5 showed that photosynthetic rate was influenced by interaction between treatments. In normal condition, or 2 days of watering interval, photosynthetic rate in all clones was not significant different. The decreasing of photosynthetic rate was accompanied by the increasing of watering interval. Watering every 5 days and 8 days declined photosynthetic rate in all clones. In severe drought stress, watering every 8 days, ICS 60 was the most suffering clone in decline of photosynthetic rate than KW 641 and Sulawesi 1 clones. Similar to Alban *et al.* (2016) that cocoa photosynthetic rate drastically declined accompanied with an increase of water stress severity.

The limitation of photosynthetic processes under drought stress is caused in consecutive ways, i.e. (a) qualitative and quantitative degradation in photosynthesizing pigments, especially chlorophyll, (b) the reduction of CO_2 uptake due to stomatal closure, and (c) low water compound because water resourches in soil and plant is limited. It was showed in Table 6. That photosynthesis rate has positive correlation with chlorophyll content, relative water content, and intercelullar CO_2 concentration. It proved that the increasing of one component will influenced in the increasing another component. In other case, photosynthesis had strongly correlation with plant height. It implied that plant height would be higher if photosynthetic rate was higher.

The foliar photosynthetic rate of higher plants is known to decrease as the relative water content (RWC) and leaf water potential decrease. However, the discussion about water deficit mainly limits photosynthesis

Tabel 4. Stomatal conductance (mmol.m^{-2}.s^{-1}), transpiration rate (mmol H_2O.m^2.s^{-1}), and intercelullar CO_2 (mmol CO_2) concentration of three cocoa clones at different watering interval treatment

Watering interval (days)	Stomatal conductance	Transpiration rate	Intercelullar CO_2 concentration
2 (normal)	0.48 a	6.02 a	343.90 a
5 (moderate stress)	0.38 a	4.39 b	346.75 a
8 (severe stress)	0.12 b	1.78 c	227.33 b
Clones			
ICS 60	0.41 p	4.72 p	317.67 p
KW 641	0.29 q	3.74 q	299.13 p
Sulawesi 1	0.28 q	3.72 q	301.18 p
Interactions	(-)	(-)	(-)

Note: Values on the same column followed by the same letter were not different based on the Tukey test with α = 5%. (-): There was no interaction.

Tabel 5. Photosynthetic rate (µmol CO_2.m^{-2}.s^{-1}) of three cocoa clones at different watering interval treatment

Clones	Watering interval (days)		
	2	5	8
ICS 60	12.43 a	6.32 bc	5.46 c
KW 641	12.30 a	9.24 b	8.53 b
Sulawesi 1	12.30 a	9.28 b	8.56 b
Interaction		(+)	

Note : Values on the same column followed by the same letter were not different based on the Tukey test with α = 5%. (+): There was interaction.

Table 6. Correlation analysis of among variables

	Chla	ChlB	ChlT	Pn	gS	Ci	E	RWC	LA	PH	SD
NR	0.64 *	0.71 *	0.69 *	0.62 *	0.39 ns	0.48 *	0.43 ns	0.65 *	0.54 *	0.65 *	0.39 ns
Chl a		0.96 *	0.98 *	0.68 *	0.53 *	0.65 *	0.56 *	0.75 *	0.65 *	0.41 ns	0.37 ns
Chl b			0.98 *	0.68 *	0.59 *	0.66 *	0.61 *	0.73 *	0.60 *	0.44 ns	0.42 ns
ChlT				0.69 *	0.57 *	0.66 *	0.60 *	0.74 *	0.62 *	0.47 *	0.40 ns
Pn					0.46 *	0.38 ns	0.57 *	0.81 *	0.88 *	0.58 *	0.38 ns
gS						0.83 *	0.95 *	0.47 *	0.26 ns	0.31 ns	0.52 *
Ci							0.84 *	0.51 *	0.29 ns	0.42 ns	0.45 *
E								0.54 *	0.38 ns	0.41 ns	0.49 *
RWC									0.83 *	0.45 *	0.41 ns
LA										0.60 *	0.38 ns
PH											0.26 ns

Note: *was significant on 5% test level; ns = non significant; NR = nitrate reductase activity, Chl a,b,T = chlorophyll a, b, & total, Pn = photosynthesis rate, gS = stomatal conductance, Ci = intercelullar CO_2, E = transpiration, RWC = relative water content, LA = leaf area, PH = plant height, SD = stem diameter.

through stomatal closure or through metabolic impairment. Photosynthesis consist of two processes, namely light reaction and dark reaction. In a light reaction that is the reaction of light energy capture. The light energy is absorbed by the thylakoid membrane and raise low-energy electrons from water molecule. The electrons move from chlorophyll a to the electron transport system that produces ATP (from ADP + P). These electrons are also captured by $NADP^+$. After receiving electrons, $NADP^+$ immediately changes to NADPH. These molecules (ATP and NADPH) temporarily store energy in the form of energy electrons that will be used to reduce CO_2. The second process of photosynthesis is dark reaction. Dark reaction is also known as Calvin cycle. In this reaction, CO_2 is converted to gliseraldehide-3-phospate. In one cycle of Calvin cycle, plant will generate 1 molecule of G3P. Thus, plant need 2 molecules of G3P (twice of Calvin

cycle process) to form glucose in photosynthesis system. Under drought stress, The reducing of CO_2 diffusion from the air to the the site of carboxylation is generally considered the main cause for reduced photosynthesis under water stress. Early biochemical effects of water deficits that involve alterations in photophosphorylation (a decrease in the amount of ATP leading to a decreased regeneration of RuBP) and seem to be dependent on species showing different thresholds for metabolic down-regulation.

CONCLUSIONS

This study concluded that drought stress reduced the growth of cocoa seedlings, mainly as a result of reducing photosynthetic activities in all stressed cocoa clone seedlings. Under drought stress, KW 641 and Sulawesi 1 had higher leaf area, chlorophyll a, chlorophyll b, total chlorophyll content, relative water content, and photosynthetic rate than ICS 60 clone.

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