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Contribution of Calcium to Changes Leaf Anatomy Character of Oil Palm Seedlings (*Elaeis quineensis* Jacq.) under Drought Stress

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ABSTRACT
The research aimed to know: (1) the effects of drought stress on changes in leaf anatomical characteristics of oil palm seedlings and (2) the contribution of calcium to cell compactness and increase in the structural strength of leaf tissue so that oil palm seedlings are more tolerant to drought stress. The experiment was arranged in split plot design with three blocks as replication. Drought stress level consisting of field capacity (FTSW 1.00), moderate drought stress (FTSW 0.35) and severe drought stress (FTSW 0.15) was used as main plot. The fraction of transpirable soil water (FTSW) was used for evaluating gradually increasing drought stress based on the amount of water loss due to transpiration. Meanwhile, subplot consists of four doses of pure calcium sulfate ($CaSO_4$) per seedling: 0.0 g, 0.04 g, 0.08 g, and 0.12 g. Leaf anatomical character were observed including epidermal length and width, hypodermal length and width, palisade cell length and width, sponge cell length and width, mesophyll tissue thickness, and xylem and phloem diameter. The results showed that moderate and severe drought stress reduced epidermal cell length, upper hypodermal cell width, mesophyll thickness, palisade width and phloem diameter of leaf vessels. The application of calcium to the leaf of oil palm seedlings under drought stresses was able to increase the sponge cell length, lower hypodermal width and diameter phloem at dose of 0.04 g/seedlings and to increase diameter xylem of leaves vessel at dose of 0.12 g/seedlings.

Keywords: Oil palm seedlings, drought stress, calcium, leaf anatomy

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is one of the plants producing main plant oil . The increasing demand of oil palm to be an attractant for oil palm producing countries especially Indonesia (Stibig *et al*., 2014) and Malaysia (Laurance, 2007) to continue the expansion of plantation areas and increase productivity.

The limited availability of fertile and productive agricultural land, especially in Indonesia, encouraged the government to expand plantations in areas that have not been extensively used as oil palm expansion (Fairhurst and McLaughlin, 2009), including areas where most of the land contain of acid soils. One of the main problems for plant growth in acid soils is the presence of Al toxicity and low avaibility of alkali cations such as Ca, Mg and K. This inhibits cations translocation to plant parts because they are strongly bound in soil particles such as Al, Fe or iron oxide is P (Pratiwi, 2014). Furthermore, leaching and weathering level of cation in acid soils is also high causing the soil cations to be easily lost. As a result, the soil becomes acidic in reaction with a low base saturation level and cations become unavailable to plants (Subagyo *et al*., 2000) causing theplants to experience nutrient deficiencies, especially Ca.

Ca deficiency in plants cuases deformities of meristem roots and shoots and dead ends of the branches (Easterwood, 2002) due to decreasing of phloem transfer and immobile Ca in plants (Gardner *et al*., 1991). This occurs because of stopped mitosis and abnormal growth in cells and plant tissues. The effect of Ca deficiency is exacerbated by the limited availability of water due to the phenomenon of global climate change. Drought stress is not only resulted from the change in physiological and biochemical processes but also from the change in the anatomical

characteristics of plants, especially plant leaves.

Although plant organs directly having contact with the limited availability of soil moisture is roots, the leaves have the earliest changes due to limited soil moisture. Disruption of division and enlargement of leaf cells due to reduced cell turgor when exposed drought stress will result in decreased leaf growth so that the process of plant metabolism is disturbed (Shao *et al*., 2008). At severe drought stress level, plant cells and tissues have irreversible changes in the form of frond fracture (Tim Peneliti Ilmu Tanah dan Agronomi, 2016).

The results of the study explaining the changes in the leaf anatomical characteristics of oil palm under drought stress are still not found. However, based on the results of Mardiyah (2014) about the effect of drought stress on the changes in the anatomical characteristics of upland rice, the upland rice experienced changes in the roots, stems, and leaves anatomy. These changes include reduced epidermal thickness, reduced endodermis thickness, reduced vascular bundle diameter, increased trichome number and reduced leaf mesophyll thickness. Similar changes also occur in the roots of soybean (Rosawanti *et al*., 2015), beans (Pena-Valdivia *et al*., 2010) and grapes (Lovisolo and Schubert, 1998) under drought stress, i.e.some changes in anatomical characteristics such as reduced cortex thickness and reduced stele and xylem diameter of the root.

When plant has not been under severe drought stress, it tries to increase the cell thickness in all organs. This cell thickness is a form of plant adaptation to drought stress so that water and nutrient use becomes more efficient. The expansion and thickening of cells stop when the availability of water is below the minimum tolerance for normal growth of plants.

One effort to reduce negative impact of drought stress on oil palm is through the addition of calcium nutrient. Calcium is a nutrient that plays a role in selective regulation and increases the integrity of cell membranes, prevents ion leakage due to environmental stresses (Naeem *et al*., 2013), composes cell walls and essential for cell division and elongation, functions as pectate in the middle lamella to strengthen cell walls and plant tissue and increases plant resistance to abiotic stresses including drought (Song *et al*., 2008). The experiment objectives were (1) to know the effects of drought stress on changes in anatomical characteristics of oil palm seedlings leaf and (2) to know the contribution of calcium to cell compactness and increase in the structural strength of leaf tissue so that oil palm seedlings are more tolerant to drought stress.

MATERIALS AND METHODS

This study was conducted from September 2017 to November 2018. Prenursery and main nursery seedling were prepared in Bendosari, Madurejo Village, Prambanan District, Sleman Regency, Yogyakarta.

This study used oil palm seedlings cv. Avross originating from the Palm Oil Research Center (PPKS) in Medan. The planting media used was latosol soil derived from the Gunung Kidul area, Yogyakarta, with soil characteristics similar to red yellow podzolic (PMK) in the original area of oil palm development. Pre nursery seedlings were put a small polybag measuring 15 cm × 20 cm and placed in a shade house made of bamboo and black paranet measuring 15 m × 5 m × 2 m with light intensity penetrated of ± 25%.

Main nursery seedlings were put in polybags large in size 40 cm × 40 cm. Seedlings were placed in plastic house using a framework of bamboo and UV plastic roofs to help maintain the temperature of the seedlings, reduce evaporation during drought stress treatment and prevent the seedlings from contaminated rainwater. The fertilizers used in this study were Urea, SP-36, KCl, Kieserit and Calcium Sulfate ($CaSO_4$) pure analysis. The fertilizers were given every two weeks until the drought stress treatment was given.

The research location has a fluctuating microclimate. Microclimate conditions during drought stress treatment including temperature, humidity and light intensity were higher than after Ca doses treatment was applied. The daily temperature during Ca treatment was 34.7 °C with daily minimum and maximum temperatures of 21.6 °C and 41.7 °C, respectively. Meanwhile, the daily temperature during drought stress treatment starting from the preparation of achieving target weights, maintenance to final sampling was 35.3 °C with daily minimum and maximum temperature of 21.6 °C and 42.4 °C, consecutively. The daily humidity and light intensity during Ca treatment and drought stress treatment were, respectively, 76.3% and 58%; and 39.900 lux and 48.190 lux.

This research was set up in split plot design with two treatment factors, i.e. drought stress as the main plot and Ca doses as the subplot with three replications.

The main plot consisted of three drought stress levels, i.e. field capacity (FTSW 1.00), moderate drought stress (FTSW 0.35) and severe drought stress (FTSW 0.15). The subplot consisted of four doses of Ca, i.e. without Ca (0 g); 0.04 g Ca (0.14 g $CaSO_4$), 0.08 g Ca (0.28 g $CaSO_4$) and 0.12 g Ca (0.42 g $CaSO_4$).

Determination of Ca requirements was carried out through analysis of Ca in plant tissues at the research preliminary stage on 4 month-old oil palm seedlings. The concentration of Ca in young leaves of oil palm seedlings after Ca doses treatment was 0.32 g (Ca 0 g), 0.45 g (Ca 0.04 g), 0.49 g (Ca 0.08 g) and 0.55 g (Ca 0.12 g). Whereas, the treatment of drought stress in this study refers to the FTSW method proposed by Ray and Sinclair (1998). Fraction of transpirable soil water (FTSW) is a method for evaluating gradually increasing drought stress based on the amount of water loss due to transpiration This method has been used by Muiz (2016) in his experiment on testing four oil palm progenies on various soil moisture levels namely field capacity (FTSW 1.00), moderate drought stress (FTSW 0.35) and severe drought stress (FTSW 0.15). The FTSW 1.0 value indicates that water is available to plants and FTSW close to 0.0 indicates that the water is not available to plants.

Calcium was given when the seedlings were considered to have passed the acclimatization period in main nursery seedlings at the age of 5 months. Meanwhile the treatment of drought stress was given when the plants were 7 months or 2 months after application of Ca.

The seedling water requirements at each of the drought stress levels were based on the polybags weight at permanent wilting point conditions. This polybag weight the permanent wilting point is a reference in determining the target weight of each polybag in drought stress trials in the field.

The weight of polybag at permanent wilting point was obtained through preliminary research using three 5 month-old oil palm seedlings. The weight of the three polybags was uniform and then the polybags were watered until reaching saturation. The seedlings were left until they reached a field capacity condition which was marked by water stops flowing from polybags. The weight of polybags at field capacity condition weighed as the weight of field capacity polybag (initial weight of polybag). The permanent wilting point of seedlings was obtained when polybag weight was constant for three days, plants wilted, a number of leaves were dry and damaged and seedlings were not refreshed even though they were turned on at 100% atmospheric humidity (Gardner *et al*., 1991). The weight of polybags at the permanent wilting points was calculated based on the final weight of polybag (SMARTRI, 2014):

$$B_{TLP} = (B_{A1}+B_{A2}+B_{A3})/3$$

Description: BTLP: Severe permanent wilting point; B_{A1-3}: Final weight of the polybag 1–3.

The next step was drought stress treatment. Polybags in each treatment combination, were weighed and saturated with water. All polybags had been saturated with water left to reach field capacity conditions. Then, the field capacity weight of each polybag was weighed and used as a reference for the initial weight of the polybag. Based on the weight of the polybag at the field capacity, then the target weight of each polybag was calculated using the following formula:

Field capacity = $1.00 (B_{KL} - B_{TLP}) + B_{TLP}$
Moderate drought = $0.35 (B_{KL} - B_{TLP}) + B_{TLP}$
Severe drought = $0.15 (B_{KL} - B_{TLP}) + B_{TLP}$

Description: BTLP: Weight of permanent wilting point on preliminary research; BKL: Weight of field capacity

Each of the polybags for moderate and drought stress treatment combination was not watered and they were weighed every day until they reach the target weight. Meanwhile, polybags for field capacity treatment combination were always watered every day until sampling. After the target weight of each polybag was reached (± 15 days), the target weight of the polybag was maintained (± 10 days). Polybags were weighed every day. If the polybag weight was less than the target weight, the polybag was watered again until it reached the target weight. All polybags in this treatment were also wrapped in plastic bags to reduce water loss due to evaporation.

Daily transpiration of plant was known from the difference in weight of today polybag with the weight of the previous day polybag. The fraction of transpirable soil water each polybag on a particular day (i) can be calculated using the following formula (Masinde *et al*., 2001):

$$FTSW = \frac{\text{(polybag weight at day } i - \text{final polybag weight)}}{\text{(initial polybag weight} - \text{final polybag weight)}}$$

where the initial polybag weight refers to the polybag weight at 100% of field capacity and the final polybag weight refers to the polybag weight when transpiration of stressed plants <10% of the well-watered plants.

The anatomical preparations of the leaves of oil palm seedlings were made by semi-permanent methods. Before slicing, the root and leaf samples were first fixed in 70% alcohol. After fixation, then a cross section was made had been given alcohol 70%. The slices were then placed in flakon bottle 25 ml which had been filled with alcohol 70% as much as ±10 ml. The next slice was observed under a microscope by magnification of 10× and 40×. The observations of the preparations were documented with Optilab then the size, length and width of each cell and leaf tissue were calculated using the Raster Image 2 application. Data were analyzed using analysis of variance (ANOVA) and DMRT at $\alpha = 5\%$. The analysis was carried out using SAS software.

RESULTS AND DISCUSSION

Leaves are plant organs that are most sensitive to water deficits. The sensitivity is shown by smaller cell and tissue size because of the reduced number of cells produced by the meristem leaves when plants are exposed to drought stress (Tardieu *et al*., 2000). This is an anatomical modification of the leaves of oil palm seedlings to survive under drought stress. By reducing cell size, the turgor pressure and the accumulation of solutes needed by cells to achieve potential growth will be lower than the large cell size which requires more turgor pressure to stretch the cell wall to begin growth.

Anatomically, the leaves of oil palm seedlings consists of epidermal and mesophyll tissue (Figure 1) and vascular tissue of xylem and phloem (Figure 2). Epidermal tissue of the leaves is differentiated into upper epidermal and lower epidermal. Epidermal plays an important role in regulating gas exchange in leaves and protecting plant tissues from outside environmental influences. Table 1 shows that there is no interaction effect between the treatment of drought stress and Ca doses on affecting the upper epidermal cell length and lower epidermal cell length of the leaves of oil palm seedlings. Individually, drought stress has a significant effect on upper epidermal cell length and lower epidermal cell length of the leaves of oil palm seedlings. The heavier the level of drought stress, the longer the leaf epidermal cell decreased. The epidermal cell length began to show a significant decrease when the seedlings were exposed to moderate drought stress and the decrease was not significantly different when the plants were exposed to severe drought stress. Meanwhile, an increase in the Ca dose of 0.12 g/seedlings did not significantly increase the upper epidermal cells length of the leaves of oil palm seedlings.

The changes in the lower epidermal cell length were seen in *Astragalus gombiformis* Pomel. under moderate drought stress (20 days) and severe drought stress (30 days) (Boughalleb *et al*., 2014).

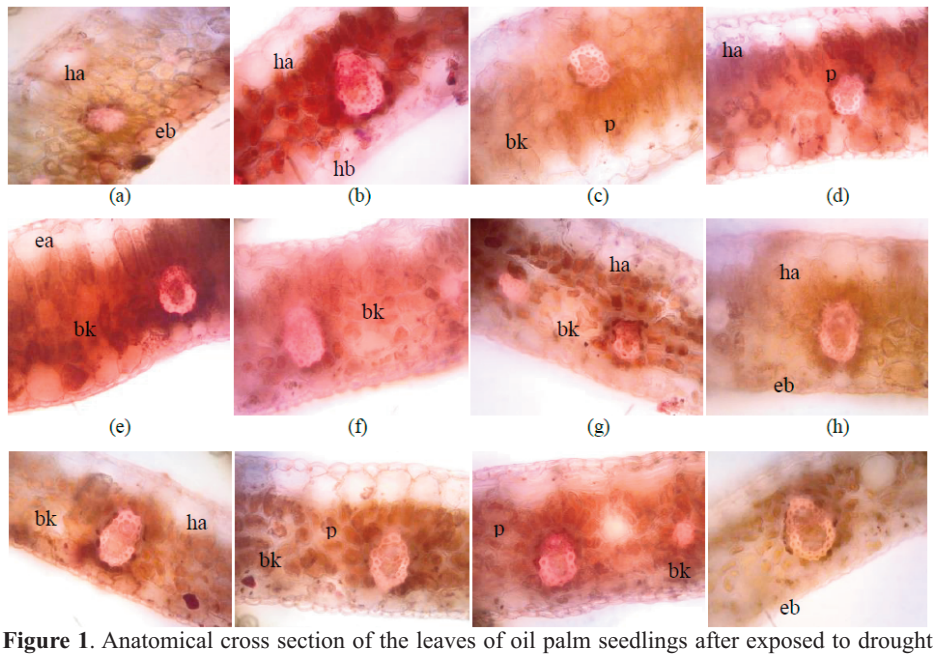

Figure 1. Anatomical cross section of the leaves of oil palm seedlings after exposed to drought stress. (A) FC: Ca 0.0 g; (B) FC: Ca 0.04 g; (C) FC: Ca 0.08 g; (D) FC: Ca 0.12 g; (E) MD: Ca 0.0 g; (F) MD: Ca 0.04 g; (G): MD: Ca 0.08 g; (H) MD: Ca 0.12 g; (J) SD: Ca 0.0 g; (K) SD: Ca 0.04 g; (L) SD: Ca 0.08 g; (M) SD: Ca 0.12 g. Abbreviation: Field capacity (FC), Moderate drought stress (MD), Severe drought stress (SD); upper epidermal (ea), lower epidermal (eb), hypodermal (h), spons tissue (bk), palisade (p). Image enlargement: 40×.

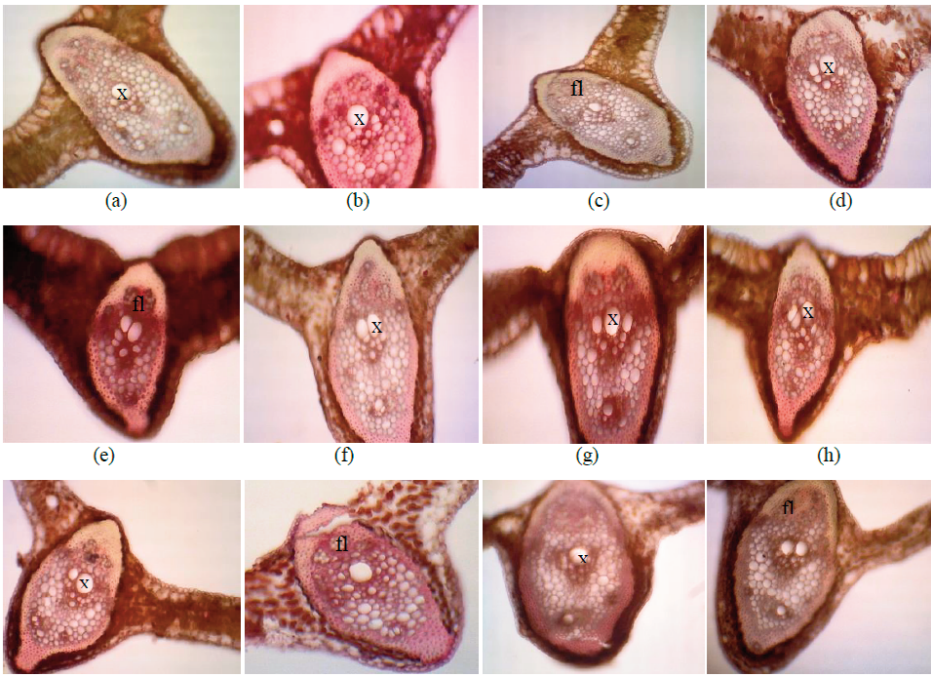

Figure 1. Anatomical cross section of vascular tissue of the leaves of oil palm seedlings after exposed to drought stress. (A) FC: Ca 0.0 g; (B) FC: Ca 0.04 g; (C) FC: Ca 0.08 g; (D) FC: Ca 0.12 g; (E) MD: Ca 0.0 g; (F) MD: Ca 0.04 g; (G): MD: Ca 0.08 g; (H) MD: Ca 0.12 g; (J) SD: Ca 0.0 g; (K) SD: Ca 0.04 g; (L) SD: Ca 0.08 g; (M) SD: Ca 0.12 g. Abbreviation: FC Abbreviation: Field capacity (FC), Moderate drought stress (MD), Severe drought stress (SD); floem (fl), xylem (x). Image enlargement: 10x.

Table 1. The anatomical character of upper epidermal cells length, lower epidermal cells length, lower epidermal cells width, hypodermal cells length and hypodermal cells width of the leaves of oil palm seedlings after exposed to drought stress

Treatments	Upper epidermal cell length(µM)	Lower epidermal cell length (µM)	Lower epidermal cell width (µM)	Hypodermal cell length (µm)	Hypodermal cell width (µm)
Drought stress					
Field capacity	12.08 a	10.14 a	11.76 a	31.32 a	28.12 a
Moderate	10.50 b	8.66 b	10.93 a	28.75 a	27.16 a
Severe	9.56 b	8.63 b	10.38 a	19.92 b	24.53 b
Calcium					
0.00 g	10.01 p	8.33 p	10.91 p	26.51 p	29.81 p
0.04 g	11.48 p	9.34 p	10.83 p	25.86 p	29.23 p
0.08 g	10.73 p	9.59 p	11.11 p	27.77 p	31.80 p
0.12 g	10.63 p	9.31 p	11.23 p	26.51 p	30.00 p
Interaction	(-)	(-)	(-)	(-)	(-)
CV (%)					
Drought stress	7.33	11.3	12.06	12.13	11.34
Calcium	13.47	13.74	9.06	14.58	13.4

Remark: Means followed by the same letters in the same column and row are not significantly different based on DMRT test at α = 5%; sign (-) indicates that there is no interaction between the factors tested.

The decreasing of the size of leaf epidermal exposed to drought stress was an effect of decreasing water availability in the growing media so that the water needed for full cell turgor was not available. Furthermore, reduced cell turgor resulted in thinner volume and thickness of epidermal cells (Taiz and Zeiger, 2002) and shrunk leaf cells, including epidermal cells. In addition, reduction of epidermal cell size in drought stress conditions is a cell defense mechanism by cell disintegration as a consequence to dry conditions.

In contrast, the width of the upper epidermal cell was affected by the interaction between drought

Table 2. The anatomical character of palisade cell width, sponge cell width, mesofil thickness and phloem diameter of the leaves of oil palm seedlings after exposed to drought stress

Treatments	Palisade cell width (μm)	Sponge cell width (μm)	Mesofil thickness (μM)	Diameter phloem (μM)
Drought stress				
Field capacity	11.58 a	19.91 a	100.82 a	8.52 a
Moderate	10.12 b	17.73 b	86.42 b	7.72 b
Severe	8.15 c	15.34 c	75.87 c	7.20 c
Calcium				
0.00 g	9.94 p	17.76 p	87.51 p	7.31 p
0.04 g	9.73 p	17.20 p	87.44 p	7.61 pq
0.08 g	9.98 p	17.60 p	88.77 p	8.14 p
0.12 g	10.13 p	18.08 p	87.08 p	8.18 p
Interaction	(-)	(-)	(-)	(-)
Coofficient of variation (%)				
Drought stress	9.22	18.23	11.26	8.22
Calcium	13.33	11.13	8.1	9.1

Remark: Means followed by the same letters in the same column and row are not significantly different based on DMRT test at α = 5%; sign (-) indicates that there is no interaction between the factors tested.

stress and Ca doses treatment (Table 3). In field capacity and severe drought stress conditions, increases of Ca doses showed a tendency towards the same effect on upper epidermal cells width of the leaves of oil palm seedlings. Increases in Ca dose of 0.08 g/seedlings and 0.12 g/seedlings significantly increased the upper epidermal cells length of the leaves of oil palm seedlings compared to control plants (Ca 0.0 g). Whereas in plants under moderate stress, increases of Ca dosage 0.08 g/seedlings and 0.12 g/seedlings did not have a significant effect on the upper epidermal cell width of the leaves of oil palm seedling. Increases of the epidermal cell width under field capacity and severe stress conditions can be attributed to the role of Ca in the maintenance and regulation of various cell functions including cell elongation. Sufficient availability of Ca^{2+} is able to increase plant tolerance to water stress (Wu *et al*., 2012). In the plant's organs, Ca mostly accumulates on the cell wall and middle lamella in the form of calcium pectate which functions to strengthen the cell wall (Taiz and Zeiger, 2002). Calcium also plays a role in increasing the integrity of cell membranes so that the membrane is more stable and inhibits the release of low molecular compounds from the cell plasma (preventing cell leakage).

Besides of the epidermal tissue, the leaves of oil palm seedlings were also composed of hypodermal tissues which have several layers of sclerenchyma cells and are impermeable to water. Hypodermal tissue functions as a barrier to water and solutes into the sclerenchyma (Esau, 1997). The effect of drought stress treatment was also seen in the hypodermal cells of the leaves of oil palm seedlings. Although the two treatment factors did not interact with each other in affecting the length and width of the hypodermal cells, individually, the increasing drought stress reduced the hypodermal cells length and hypodermal cells width of the leaves of oil palm seedlings. The decreased soil moisture content in severe drought stress caused the hypodermal cells of the leaves to be shorter. In contrast to the effect of Ca, the increase in Ca dose up to 0.12 g/seedlings did not have significant effect on the length and width of the hypodermal cells of the leaves of oil palm seedlings.

Between the upper and lower epidermal tissues of the leaves of oil palm seedlings, there are also mesophyll tissue. The mesophyll tissue has thin and chloroplast rich cell walls that function for photosynthesis. Similar to other leaf organs, the mesophyll thickness is also influenced by water availability but is not affected by increase in Ca doses (Table 2). The decreasing availability of groundwater under moderate and severe drought stress lowered the leaf mesophyll thickness. This is a result of the decreasing availability of nutrients and water that can be absorbed so that plants must balance the photosynthesis process when exposed to drought stress. In addition, the palisade and spongy cells that make up the mesophyll tissue under drought stress are also smaller than under the field capacity conditions (Chartzoulakis *et al*., 2002). The same effect was

seen in Ctenanthe setosa and Triticum aestivum, in which all lamina and mesophyll thickness decreased under drought stress (Kutlu *et al*., 2009; Burnett *et al*., 2005).

Some of the study results also showed that the size of upper epidermis cell, lower epidermis cell and mesophyll thickness in legume under drought stress were significantly reduced (Chartzoulakis *et al*., 2002). The decrease in mesophyll thickness was also seen in Astragalus gombiformis Pomel. exposed to drought stress for 10 days, 20 days and 30 days. The mesophyll thickness of Astragalus gombiformis Pomel. decreased significantly when exposed to moderate and severe drought stress (20–30 days) (Boughalleb *et al*., 2014). This is a leaf mechanism allowing CO_2 to enter the chloroplast in the palisade tissue at the time of the opening of the reduced stomata and a plant strategy to protect photosynthesis when the plant is exposed by drought stress.

The leaf mesophyll tissue also consists of palisade and sponge tissue. The sponge tissue also has chloroplasts but not as many as in the palisade tissue. The two mesophyll constituent tissues also showed changes in anatomical character due to drought stress and Ca doses treatment. In Table 3, it can be seen that there is an interaction between drought stress and Ca doses affecting the length of leaf palisade cells. The increase of Ca dose to 0.12 g/seedlings did not have significant effect on the length of palisade cells under field capacity and moderate drought stress conditions. However, in oil palm seedlings exposed to severe drought stress, an increase in the Ca dose to 0.04 g/seedlings and 0.08 g/seedlings increased the palisade cells length. When the Ca dosage was increased to 0.12 g/seedlings, the palisade cells length decreased and was not significantly different from that treated with Ca dose of 0.08 and 0.00 g/seedlings.

In contrast to palisade cells length, drought stress significantly decreased the leaf palisade cells width (Table 2). The heavier the level of drought stress, the smaller the palisade cells width. Meanwhile, an increase in Ca dose to 0.12 g/seedlings was not able to increase the width of leaf palisade cells significantly.

Ca oxalate is generally stored in leaf mesophyll especially in palisade tissue. The presence of Ca accumulation on the palisade has an impact on increasing the length of palisade cells. Increasing the length of palisade cells when exposed to drought in the presence of Ca is expected to increase the

Table 3. The characteristics anatomy of upper epidermis width, palisade length, sponge length and xylem diameter of the leaves of oil palm seedlings after exposed to drought stress

Treatments	Upper epidermis width (µm)	Palisade length (µm)	Sponge length (µm)	Xylem diameter (µm)
Field capacity - Ca 0.00 g	13.83 b	32.57 a	21.47 a	39.60 bcd
Field capacity - Ca 0.04 g	11.80 bcd	29.07 ab	18.43 b	41.60 abcd
Field capacity - Ca 0.08 g	17.53 a	33.63 a	19.27 ab	36.27 cd
Field capacity - Ca 0.12 g	16.37 a	34.30 a	17.37 bc	44.03 abc
Average	14.88	32.39	19.14	42.33
Moderate - Ca 0.00 g	12.53 bc	33.17 a	19.17 ab	32.53 d
Moderate - Ca 0.04 g	12.17 bcd	29.90 ab	16.23 bcd	47.87 ab
Moderate - Ca 0.08 g	11.77 bcd	22.60 bc	14.43 cd	48.80 ab
Moderate - Ca 0.12 g	11.10 cd	28.40 ab	16.43 bcd	42.03 abc
Average	11.89	28.53	16.57	42.8
Severa - Ca 0.00 g	9.90 d	13.43 d	7.77 f	39.83 bcd
Severa - Ca 0.04 g	11.00 cd	23.57 bc	14.30 d	36.70 cd
Severa - Ca 0.08 g	12.37 bc	17.13 cd	10.87 e	39.70 bcd
Severa - Ca 0.12 g	12.70 bc	15.10 d	10.53 e	49.32 a
Average	11.27	17.29	10.87	45.52
Interaction	(+)	(+)	(+)	(+)
Coofficient of variation (%)				
Drought stress	5.4	15.47	23.07	4.97
Calcium	9.51	15.26	10.22	11.64

Remark: Numbers in one column and row followed by the same letters show o significant difference based on DMRT test level of α = 5%; sign (+): there is an interaction between the factors tested.

amount of diffusion of CO_2 bound by leaves to help maintain the rate of assimilation and stomatal conduction (Ennajeh *et al*., 2010).

Changes in leaf anatomical character was also indicated by the length and width of the sponge cells. The data in Table 3 showed that when soil moisture was in a condition of field capacity and moderate drought stress, an increase in Ca dose tended to decrease the sponge cells length even though the decrease was not significant at Ca dose of 0.04 g/seedlings, 0.08 g/seedlings and 0.12 g/seedlings. Otherwise, under severe drought stress, the sponge cell length increased when the plant was given Ca 0.04 g and when the dose of Ca was raised to 0.08 g/seedlings and 0.12 g/seedlings, the sponge cell length tended to decrease and was not significantly different.

The shortest sponge cells were shown by plants that were not given Ca (Ca 0.0 g) before being exposed to severe drought stress. In contrast, there was no interaction between drought stress and Ca doses in influencing the sponge cells width (Table 2). Individually, drought stress had significant effect on the sponge cells width. The sponge cell width decreased with the increasing drought stress. Meanwhile, an increase in the Ca dose to 0.12 g/seedlings did not show significant increase in the sponge cells width.

The xylem and phloem tissue leaves of palm oil seedlings are also influenced by drought stress and Ca doses (Table 2 and Table 3). The increase in Ca doses in a plant with field capacity conditions did not show a significant effect on the xylem diameter of leaf vessels (Table 3). In moderate drought stress, an increase in the dosage of Ca 0.04 g per seedling and 0.08 g per seedling was able to increase the xylem diameter of the leaf vessels. If the Ca level is increased to 0.12 g per seedling, the xylem diameter tends to decrease, even though at these three levels the xylem diameter is not significantly different. The lower soil moisture, until it reaches severe drought stress, causes the addition of Ca dosage 0.12 g per seedling significantly increases in the xylem diameter.

During drought stress, water tension in the leaf xylem vessels will increase so that it can lead to xylem embolism. The plant will close the stomata to prevent loss of xylem conductivity. Even after stomata closure, the plant slowly loses water through the stem and tissue cuticles because the closure of the stomata does not completely prevent embolism but significantly reduces the rate of water loss and embolism. Increasing availability of Ca under stress will help plants in the lignification process so that the xylem diameter will be larger. Thus, the transport of water from roots to leaves can increase. In regulating cell membrane permeability, Ca is also associated with potassium (K). Availability of K sufficiently increases the permeability of cell membranes through increased absorption of water into cells. Whereas, Ca helps increase water release from cells so that transpiration and the rate of water and nutrients absorption through mass flow increases.

Phloem also plays a role in transporting photosynthesis products to other plant organs. The wider the phloem diameter, the more efficient the assimilate process. Table 2 shows that there is no interaction between drought stress and Ca doses affecting the phloem diameter. Decreasing availability of water under moderate and severe drought stress significantly decreases the phloem diameter. The reduction in phloem diameter is due to disruption of cell rigidity and decreased cell turgor so that the cell shrinks and the process of division and elongation of phloem cells is disrupted (Patakas *et al*., 2002).

In contrast to the effects of drought stress, an increase of the Ca dose 0.04 g per seedling began to increase the phloem diameter, even though the increase was not significantly different from that in Ca dosage 0.00 g per seedling. When the Ca doses was increased to 0.08 g per seedling and 0.12 g per seedling, the phloem diameter appeared to be enlarged and significantly exceeded the phloem diameter of the oil palm seedlings that were not given with Ca (Ca 0.00 g). The increase in phloem diameter illustrates an increase in the elongation and enlargement activity of phloem cells after oil palm seedlings are given sufficient amounts of Ca.

During cell division, Ca plays a major role in the anaphase stage. Calcium is concentrated in spindle poles which causes depolymerization of microtubules (Hepler, 2005). When entering the prophase stage, the core membrane is disintegrated into vesicles. Following anaphase, a new cell wall is formed. During this process, Ca is very necessary to stabilize the developing cell wall by binding to pectate (Robertson, 2013).

CONCLUSIONS

Based on the results of this research it can be concluded that moderate and severe drought stress decreased the size of the leaf cells and tissue of oil palm seedlings, decrease in the length of upper and lower epidermal cells, the width of upper hypodermal cells, the length of lower hypodermal cells, the

thickness of mesophyll, the width of palisade and phloem diameter of the leaf vessels. The applications of Ca to oil palm seedlings under drought stresses were able to strengthen cell compactness and increase the structural strength of the leaf cells. The leaves of oil palm seedlings were increased in the length of sponge cells at Ca dose 0.04 g per seedling; in the width of lower hypodermal and diameter of vessel phloem at Ca dose 0.08 g per seedling; and increased in the xylem diameter of the leaves of oil palm seedlings at Ca dose 0.12 g per seedling.

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