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Research Article
Physiological Character of Palm Oil Seedling due to Lack of Water with Bokashi Treatment

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Abstract

Background and Objective: The problem of drought is a scourge for oil palm farmers in Indonesia, the development of oil palm plantations is directed to areas that have dry land potential with a dry climate, longer dry months and lower rainfall. The purpose of this study was to determine the physiological character of oil palm seedlings due to water stress and Bokashi treatment. **Materials and Methods:** The experiment was conducted in June to September, 2021. The experiment used a non-factorial completed randomized design with 9 treatments and 2 replications. The observed variables were leaf chlorophyll, total protein, total sugar and *p5CS* gene expression level by the qPCR method. **Results:** The results showed that the less water, the smaller the leaf chlorophyll, while the total sugar and total protein tended to be higher and the *p5CS* gene expression level by qPCR method activity under water stress conditions of 14.58 was higher than under conditions of sufficient water. **Conclusion:** The physiological character of oil palm seedlings differs in the treatment with sufficient water and lack of water.

Key words: Physiological character, oil palm, seedling, water stress, sufficient water, Bokashi, *p5CS* gene expression

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Abiotic stress certainly reduces plant growth, development and productivity. Drought-tolerant plants are regulated by a complex network of genes where microRNA plays an important role in drought-tolerant plants[1], in this case, there is a role of micro RNA in drought tolerance[2] plants. On a plant, overexpression of certain genes stabilizes a set of mRNA to increase ATP production so that it is more tolerant to drought[3]. So it is clear that the high *p5CS* gene expression level by qPCR method activity is maintained to support protein synthesis[4]. The PCR method is very efficient, simple and is an easy technique for measuring gene expression. From previous studies, it was also found that only 40-44% of transcripts would participate in the drought stress response as functional proteins[4-9]. Even longer RNA activity in drought-tolerant pea plants (identification of drought)[1,4,10]. Drought tolerant transgenic rootstocks can be developed[1,3,4,11].

The problem of developing oil palm cultivation in dry areas can no longer be avoided because climate change has become a reality today, while physiological characteristics due to lack of water are very important to know as scientific capital for development in dry areas.

The objective of this study was to determine the physiological character of oil palm seedlings due to water stress and Bokashi treatment, namely leaf chlorophyll, the total sugar and total protein and the *p5CS* gene expression level by the qPCR method.

MATERIALS AND METHODS

Study area and sample collection: After the seedlings are obtained from the PPKS, the sprout bags are carefully removed and placed in a shallow tray filled with water to keep the sprouts fresh. The current research used the Tenera Simalungun variety obtained from PPKS (Pusat Penelitian Kelapa Sawit, Medan, Indonesia) and the experiment was carried out in a plastic house to determine several physiological characteristics. This experiment was conducted in June to September, 2021.

Methodology: Current study used a non-factorial completely randomized design with 9 treatments namely:

- B_1W_1 (Bokashi 45 g/polybag+daily watering)
- B_1W_2 (Bokashi 45 g/polybag+watering 2×1 week)
- B_1W_3 (Bokashi 45 g/polybag+watering 1×1 week)
- B_2W_1 (Bokashi 30 g/polybag+daily watering)
- B_2W_2 (Bokashi 30 g/polybag+watering 2×1 week)
- B_2W_3 (Bokashi 30 g/ polybag+watering 1×1 week)
- B_3W_1 (Bokashi 15 g/polybag+daily watering)
- B_3W_2 (Bokashi 15 g/polybag+watering 2×1 week)
- B_3W_3 (Bokashi 15 g/polybag+watering 1×1 week), each treatment was repeated 2 times

Experimental variables observed at 56 days after planting included: Total leaf chlorophyll, total sugar, total protein and *p5CS* gene expression level by qPCR method. To measure leaf chlorophyll using a spectrophotometer by following the procedures carried out by Latifa *et al.*[12]. To measure procedures of total protein content are similar to those worked by Sarkar *et al.*[13].

In the calculation of total sugar, after the supernatant was heated to 85°C, 3-5 mL was transferred to a measuring flask and then added with distilled water to 50 mL, then 2 mL was taken and 4 mL of anthrone was added, heated to 85°C for 15 min, then removed and cooled on ice and then read at a wavelength of 630 nm. The next procedure is as done by Islam *et al.*[14].

After DNA isolation, the next several steps are gene expression validation using RT-qPCR using the *P5CS* target gene. The primers used are F: 5' CGGTTGGAAGATTGGGAGCT 3' and R: 5' TTGGGGTTTCTGAAGGTCGG 3', RT-qPCR conditions according to the procedure holding stage 95°C 2 min, cycling stage 45×cycles 95°C 5 sec, annealing 58°C 30 sec. Melting stage 95°C 15 sec, step and hold 60°C 1 min. This follows the procedure previously worked by Turhadi *et al.*[5].

Statistical analysis: An analysis of variance was used data processing, was used for data processing and if the treatment was significant then Duncan's average difference test at the 5% level was done. Response variables were observed in USU and other places.

RESULTS

The result of Duncan's mean difference test as a continuation of the results of the analysis of variance can be seen in Table 1.

Leaf chlorophyll from Table 1, it was found that B_1W_1 (Bokashi 45 g/polybag+daily watering l) produced the highest leaf chlorophyll followed by B_1W_2 (Bokashi 45 g/polybag+watering 2×1 week) which was not significantly different while the lowest total chlorophyll in the B_3W_3 treatment (Bokashi 15 g/polybag+watering 1×1 week). Furthermore, the highest total protein was produced

Table 1: Chlorophyll content, total protein, total glucose and *p5CS* gene expression level by qPCR method

Treatments	Total leaf chlorophyll	Total protein (% dw)	Total glucose (%) (cg/cg)	*p5CS* gene expression level by qPCR method
B_1W_1	18.16^{ef}	4.68^{a}	61.00^{a}	1.00
B_1W_2	18.15^{ef}	4.69^{a}	62.00^{a}	
B_1W_3	18.08^{ef}	4.80^{ab}	63.00^{a}	
B_2W_1	16.58^{de}	4.90^{b}	66.00^{ab}	
B_2W_2	15.01^{cd}	4.91^{bc}	68.00^{abc}	
B_2W_3	13.38^{bc}	4.93^{bc}	71.00^{abc}	
B_3W_1	17.20^{def}	4.96^{bc}	73.00^{abc}	
B_3W_2	12.88^{b}	5.11^{cd}	80.00^{bc}	
B_3W_3	9.825^{a}	5.16^{d}	83.00^{c}	14.58

Numbers followed by the same letter in the same column are not different from the LSD test at the 5% level

by B_3W_3 followed by B_3W_2 treatment, while the smallest total protein was produced by B_1W_1 treatment. The highest total glucose was also produced by the B_3W_3 treatment, followed by the B_3W_2 treatment while the B_1W_1 treatment produced the smallest total glucose.

From Table 1 it can also be seen that the B_1W_1 treatment produced a *p5CS* gene expression level by qPCR method only 1 time, while the B_3W_3 treatment was 14.58.

DISCUSSION

In general, the results of the study revealed that when oil palm seedlings lack water, the total leaf chlorophyll decreases, whereas the total protein and total glucose increase. These results are not much different from the results of previous studies, including for other commodities[4-10] and the expression of genes related to the formation of osmoprotectants is increased[5-7]. From the results of the study, it can be seen that the role of Bokashi is very important, in the future to a certain extent it can be used to reduce the negative impact of water shortages. Likewise, gene activity in water shortages, *p5CS* gene expression level by qPCR method increased 14.58 times more actively than with sufficient water as recommended[4-10]. Decreased water supply causes a water deficit[15-17] and has been extensively studied in leaves[18-20]. Drought causes changes in turgor pressure, osmotic pressure and water potential. Thus, drought will cause cell shrinkage, a decrease in cell volume and a decrease in turgor pressure so that it inhibits growth, meaning that the enlargement and elongation of cells are disrupted so that leaf expansion is inhibited, by itself the number of chlorophyll decreases. In addition, it can also increase the viscosity of cell fluid, resulting in protein aggregation and denaturation as well as an increase in the concentration of solutes, which in excess can be toxic. Drought also destroys the chlorophyll pigment and deterioration of thylakoid function[19]. Bokashi is only able to support the formation of chlorophyll with the availability of water and is less functional if there is a lack of water, on the other hand, total protein and total glucose increase with watering once per week. Bokashi is organic material rich in biological sources with a fermentation process of organic matter using EM4 technology (effective microorganism 4). According to information from the producer, EM4 contains *Azotobacter* sp., *Lactobacillus* sp., yeast, photosynthetic bacteria and cellulose-decomposing fungi, so it is strongly influenced by the ratio of the C/N raw materials used. As is known, the main functions of chlorophyll in the photosynthesis process, namely utilizing solar energy and triggering CO_2 fixation to produce carbohydrates. One of the factors that influence the formation of chlorophyll in water so if there is a lack of water, the formation of chlorophyll will be inhibited[19-21]. This research complements and enriches the repertoire of knowledge, especially the physiological character which is very useful for the educated. Knowing gene expression quickly in palm oil seedlings with the qPCR method on the treatment lack of water. With a lack of water, the physiological character of oil palm seedlings can be predicted. It is not known whether the results of this study apply to all varieties of oil palm and in various places in Indonesia.

CONCLUSION

The less water, the smaller the leaf chlorophyll, while the total sugar and total protein tended to be higher and the *p5CS* gene expression level under water stress conditions of 14.58 was higher than under conditions of sufficient water.

SIGNIFICANCE STATEMENT

This experiment discovers the physiological characteristics of palm oil seedlings due to a lack of water. In the future, it is suspected that the use of Bokashi can reduce the severe negative impact of water stress. Thus, a new theory is found that the less water, the smaller the leaf chlorophyll, while the total sugar and total protein tended to be higher

and the *p5CS* gene expression level by qPCR method activity under stress conditions of 14.58 was higher than under conditions of sufficient water, all response physiological character can different between water stress condition and sufficient of water may be arrived.

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