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# Colorimetric Technique for Monitoring Water Stress in Palm Oil Seedlings

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**Abstract.** Prevention of stress in the asymptomatic stage of the plants could result in improved crop management. In this study, the water and light stress of three oil palm seedlings (*Elaeis Guineensis*) was examined by assessing the leaves' colour using CIELAB colour space. The oil palm seedlings were subjected to water stress for 33 days and then to water and light stress from 55 to 78 day (for 25 days). The variation of the colour of the leaves due to water stress was discussed in detail. The approach used in this study to identify the drought stress may allow for differentiating mild environmental and severe drought stress in oil palm plants and may be used for remote field-scale estimation of plant stress resistance and health.

## 1. Introduction

Plants are considered extremely substantial for humans and other lives on Earth. Water is an essential molecule in all physiological processes of the plant since it is the essential carrier for transporting metabolites and nutrients. Water stress occurs in two cases: if the water provides to a plant's root is scarce or the rate of transpiration is extreme. The plant water stress is one of the key factors for final harvest and is usually detected only after it becomes visually evident [1]. Drought is defined as a condition in which plant water potential and turgidity are lowered to the point where the plant is incapable to perform essential physiological tasks [2]. Plant responses to droughts are challenging, including destructive effects and adaptive changes, and can be influenced by other stresses. Water stress can range from mild and short-term droughts to severe and long-term hot weather droughts that have a significant influence on the progress and living of plants [3]. The responses of the plant to water stress are varied depending on species, soil type, nutrients, and weather [4]. The presence of water stress can be recognized by a drop in moisture of the soil, consumption of the root zone soil moisture, and the physiological response of the plants [5].

Light is another significant factor for plant growth and development. Generally, daylight is the source of light for photosynthesis to occur [6]. Light has a complex impact on plant-biotic interactions, affecting both light-intensity-dependent photosynthetic processes and mechanisms that respond to light quality and duration. It determines the photosynthetic rate and accumulate-assimilation, growth, and yield of the plant. The plants are frequently exposed to extreme or deficient light intensities, affecting growing conditions by constraining physiological metabolic processes. As a result, the plants have evolved a variety of protection and response mechanisms to alleviate the impact of light stress [7].

Palm oil is the second most traded vegetable oil crop in the world after soybeans [8]. Malaysia and Indonesia represent 85% of the world's palm oil supply. Malaysia is one of the largest producers and



exporters of palm oil in the world [9]. Palm oil is a tropical palm tree therefore it can be cultivated easily in Malaysia. Palm oil not only can be used as a source of edible oil but also can be enhanced into excellent renewable energy. In the past, the disease was only detected in palm trees at a late stage, but it is now also discovered in young palms, causing symptoms to show up earlier [10, 11]. Prevention of disease and stress, while the plants are still in an asymptomatic stage could lead to better crop management in agricultural industries.

Environmental factors such as light and water are abiotic factors of plant stress. Using the leaf to study the plant's water and light stress may provide a better picture of the plant's physiological response. In recent years, various non-invasive methods have been developed for biomonitoring stress constraints in plants. The colorimetric method using the CIELAB colour system has been used in various agriculture studies. Ahmed et al. [12] showed that the combination of the colour parameters of the CIELAB system can be used to describe the colour degradation in green chilli puree. Medeni Maskan [13] used the colour parameters of CIELAB to measure the colour difference between the fresh and dried bananas. Elçin et al. [14] studied the effect of microwave output power and sample amount on the colour kinetics of celery leaves. They showed that the microwave heating process resulted in a colour shift towards a darker region. Mola et al. [15] measured the changes in the colour of fresh-cut rose apples using the colorimetric method and CIELAB colour system. Tao et al. [16] showed that using the colour difference of CIELAB, the colour levels of rice leaves can be distinguished. Lipan et al. [17] investigated the peel colour of mango samples during the ripening process by colourimeter and CIELAB colour coordinates.

Indeed, the decrease of photosynthesis as a response to plant stress causes changes in the reflectance and colour of the leaves [18]. The chromaticity parameters of the leaves are related mathematically to the response of plants to stress factors. This study aims to evaluate the potential of the colorimetric technique to identify the colour variation of leaves caused by water and light stress in palm oil seedlings. An interpretation of colorimetric alteration that is directly connected to the health conditions of a plant could help the final user in understanding the stress factors in palm oil seedlings.

## 2. Materials and Methods

Palm oil seedlings with an age of approximately 2-3 months were purchased from AEON Nursery located in Changlun, Kedah, Malaysia. All samples are produced locally, and their characteristics are subjected to environmental conditions and weather in Malaysia. A total of three palm oil seedlings were used in this study. Plants 1 and 2 were subjected to water stress for 33 days (means the plants were not daily watered) and then water stress and light stress from 55 to 78 day, while plant 3 was watered daily (considered as control) and then subjected only to light stress. All the plants were located under a shade with average daily sunlight exposure of 4.48 Klux during the water stress treatment and 27.15 Klux during water and light stress. Daily mean values of the chromaticity parameters were recorded using a hand-held colorimeter (Model WR-10 QCR) portable colour analyser for each plant and treatment (unwatered and well-watered). The portable colour analyser used CIELAB colour space, illumination condition of CIE, light source D65, sensor photodiode array, and CIE 10° standard observer. The colour parameters values were measured in a room with controlled light environment. The device was calibrated before the tests with a white ceramic plate. Three duplications of each test were achieved, and the data given is an average of the three measurements.

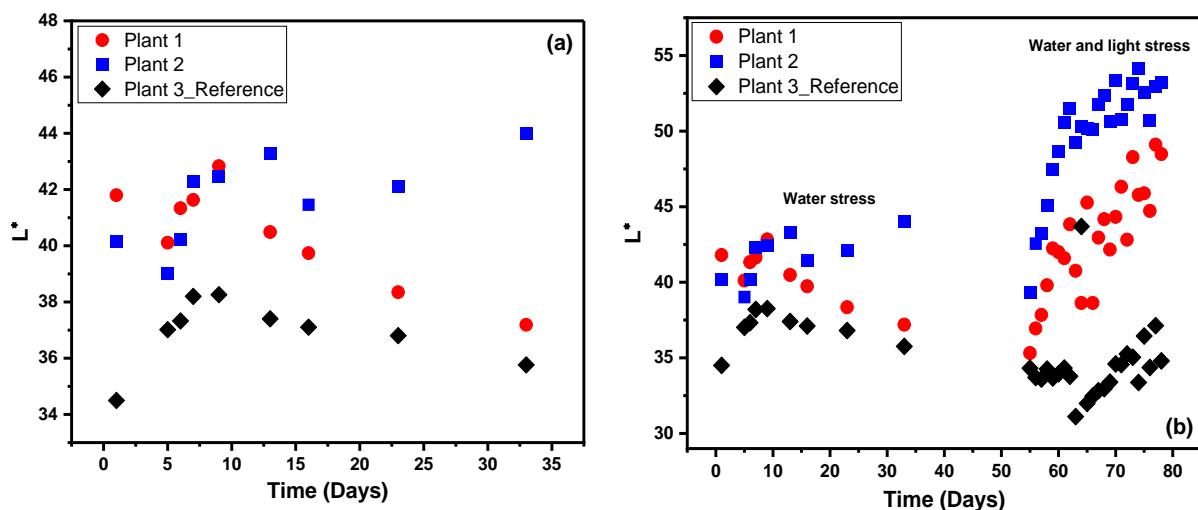
## 3. Results and Discussion

The trend of the colour changes over time for the palm oil plants is analysed and discussed in this section. Plants 1 and 2 were subjected to water stress for 33 days and then water stress and light stress from 55 to 78 day, while plant 3 was watered daily (a reference) and then subjected only to light stress. The average of the colour parameters  $L^*$ ,  $a^*$ , and  $b^*$  values of two leaves for each plant was calculated and presented in Figures 1-3. " $L^*$ " represented the lightness as a value from 0 to 100 for black to white,

respectively. The “ $a^*$ ” and “ $b^*$ ” values indicate the chromaticity coordinates. Green to red is expressed by the  $a^*$  value. A more positive and negative value signifies red and green, respectively. Blue to yellow is expressed by the  $b^*$  value. A more positive value means yellow, and a more negative value means blue.

It was showed that the colour of the three plants was influenced irrespective of the stress factor. As presented in Figure 1, in general, lightness values were lower for plant 3 (daily watered plant). The lightness decreased with water stress time as presented in Figure 1a for plants 1 and 3. Since the  $L^*$ -value is a measure of the colour in the light-dark axis, this dropping value implies that the samples were becoming darker. After 33 days of water stress, the values of  $L^*$  for plants 1, 2, and 3 were 37.19, 44.0, and 35.76, respectively. On the other hand, after 78 days of water and light stress, the values of  $L^*$  were 48.48, 53.23, and 34.80 for plants 1, 2, and 3, respectively. As can be seen from Figure 1a, there is a small variation in the lightness ( $L^*$  value) for well-watered plant leaves (from 34.5 to 35.8), while a clear fluctuation in the  $L^*$  value and a wide change in colour of unwatered plant leaves can be observed (plant 1: from 41.8 to 37.2 and plant 2: from 40.2 to 44.0).

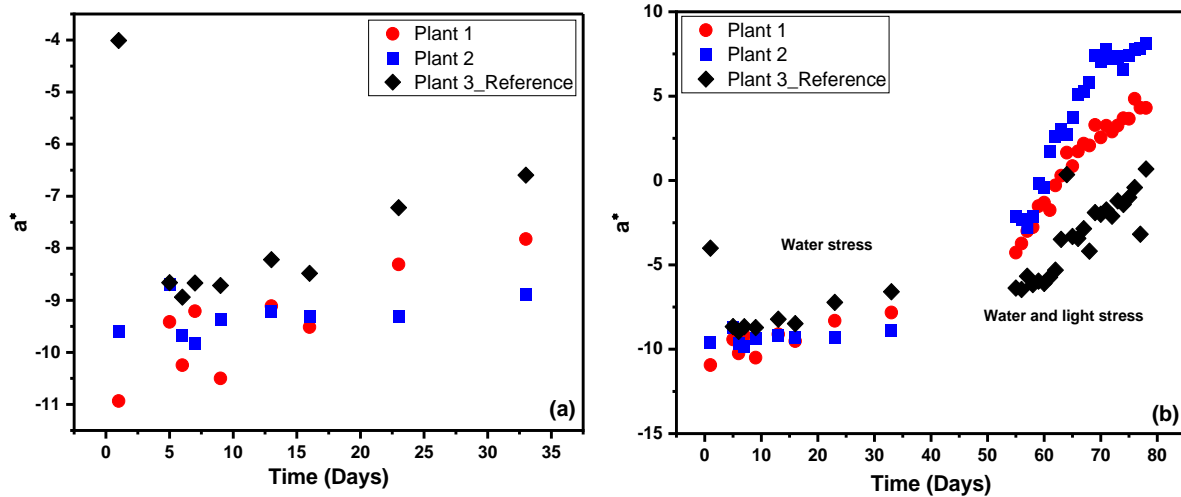
As shown in Figure 1b, when the three plants were subjected to water stress combined with light stress after 55 days, the values of lightness for plant 3 (only subjected to light stress) are still less than the unwatered plants (plants 1 and 2). It is obvious that the colour parameter  $L^*$  represents high values of lightness for plants 1 and 2 which is the same as the human visual perception, the colour is getting lighter. The lower values of  $L^*$  for plant 3 showed low lightness which means looks darker. Therefore, it can be decided that the plants subjected to water stress combined with or without light stress showed higher values of  $L^*$  compared to the daily watered plant.



**Figure 1.** Lightness changes of oil palm seedlings leaves with time due to (a) water stress and (b) water and light stress.

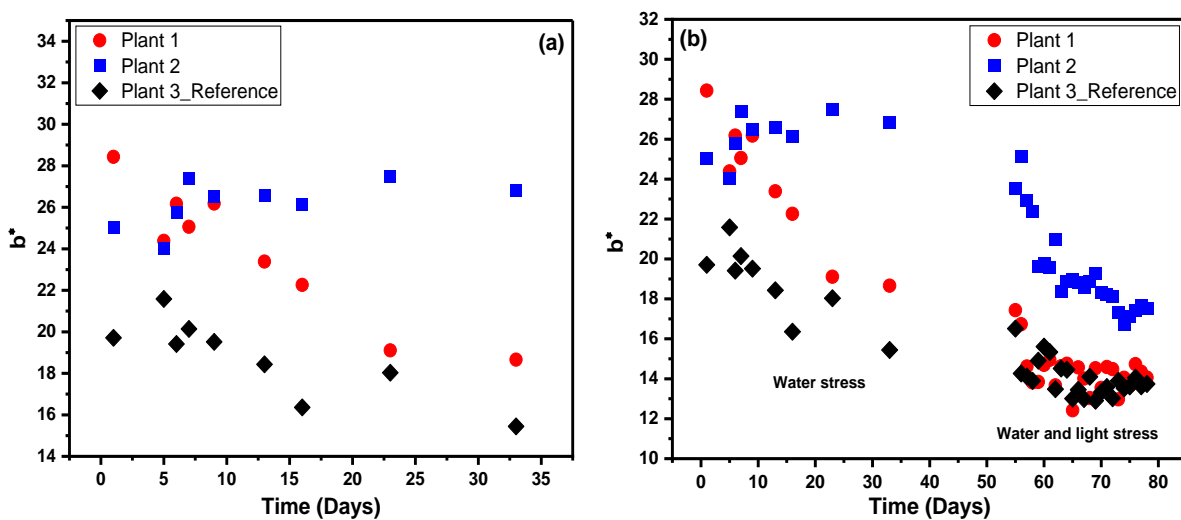
From Figure 2, the colour coordinate  $a^*$  is significantly changed due to the water and light stress from green values ( $a^* < 0$ ) to the red values ( $a^* > 0$ ) for plants 1 and 2 with an intermediate  $L^*$  of 42.16 and 47.59, respectively. Plant 3 presented a clear trend toward green colour with an intermediate  $L^*$  value of 35.01 as presented in Figures 2a and b. From Figure 2a, in the case of water stress treatment, the samples showed a negative  $a^*$ -value which was -7.82, -8.89, and -6.59 for respectively plants 1, 2, and 3 on day 33, representing greenness. It was clear that the parameter  $a^*$  achieved values more than +3.0 when subjecting plants 1 and 2 to water and light stress (+4.30 and +8.11) which means they lost their greenness and yellowness and turn out to be redder. A colour shift toward positive  $a^*$ -direction showed more redness during water and light stress of palm oil plants. This can be because of the decay

of chlorophyll and carotenoid pigments and the development of brown pigments [13, 19-23]. This change was manifested by a rise in  $a^*$ -value (Figure 2) and a decline of the  $b^*$ -value (Figure 3).



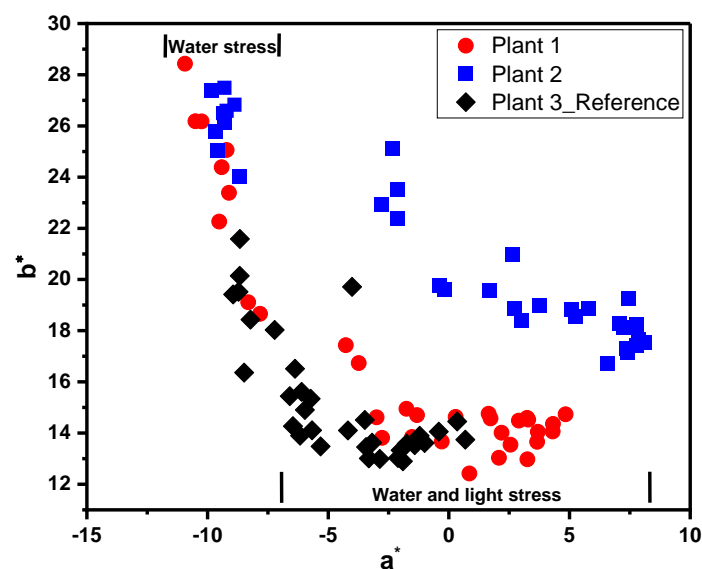
**Figure 2.** Changes of  $a^*$  colour coordinate of oil palm seedlings leaves with time due to (a) water stress and (b) water and light stress.

As exhibited in Figure 3, the values of colour coordinate  $b^*$  which denotes blue colour ( $b^* < 0$ ) and yellow colour ( $b^* > 0$ ) changed completely from day 1 to day 33 because of water stress. Obviously, there is a difference between watered and water-stressed plants. The lowest values of  $b^*$  coordinate at the commencement and end of the water stress period (33 days) for plant 3 means that this plant has the lowest yellowish remarks. However, in the case of water and light stress (Figure. 3b), all plants showed a similar trend, a decline of  $b^*$  coordinate values. Plants 1 and 3 have approximately the same values of  $b^*$ , however, plant 2 showed the highest  $b^*$  values and more decline in  $b^*$  values with time. The changes of  $L^*$ ,  $a^*$ , and  $b^*$  values due to the water stress of the three plants indicated that the method used in this work was effective.



**Figure 3.** Variations of  $b^*$  colour coordinate of oil palm seedlings leaves with time due to (a) water stress and (b) water and light stress.

Due to the overlapping of the  $b^*$  values for plants 1 and 3 during the time of water and light stress, the relation between  $a^*$  and  $b^*$  parameters are plotted as shown in Figure 4. It is apparent that, depending on  $L^*$ , the colour of the leaves of the plants under water stress (plants 1 and 2) and daily watered plant (plant 3) were clearly distinguished from each other. Additionally, there is a slight change in the greenness (negative  $a^*$ )/yellowness (positive  $b^*$ ) in plant 3 compared to other plants when they were subjected to water and light stress. This means that the water stress combined with light stress highly affects the colour of the leaves compared to light stress alone. Therefore, the results showed that there is a reduction in the colour of the leaves of the young oil palm plants due to the water stress, and the highest drop in the colour was obtained when the same leaves were subjected to water and light stress. The findings showed in this study indicate that the difference in  $L^*$ ,  $a^*$ , and  $b^*$  may participate considerably to the perception of the colour alteration.



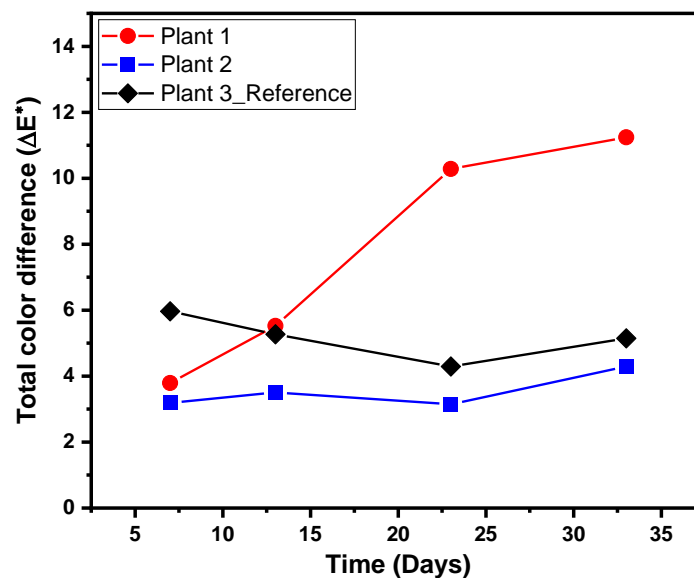
**Figure 4.** ( $a^*$ ,  $b^*$ ) colour space of oil palm seedlings under water and light stress.

It must be pointed out that the reaction and severity of plants due to stress could differ with different plants [24, 25]. For example, some plants could survive a long time without water, while some will dry out within just a few days. This can explain the difference in colour changes with time for the plants under water stress (plants 1 and 2) and also the similar behaviour of colour changes between plant 1 (unwatered plant) and plant 3 (daily watered plant). In addition, the water stress might result in closure of leaf stomates and decline in  $\text{CO}_2$  source. Thus, the change in the colour in drying leaves can be assigned to a combination impact of the variation of the leaf structure and the light absorption by photosynthetic pigments. Furthermore, leaf turgor is associated with cellular growth and function. When turgor becomes zero under great water lack, cells damage, and the leaf wilts. The turgor can be maintained by cell wall hardening during the increase of the water deficiency. While cell wall hardening aids to maintain turgor, it inhibits cell growth. This is only one of several instances where the protection of plant water status under drought stress is relatively attained by growth drop. Therefore, both a decline in the progress of the plant and variations of the leaves structure are the principal factors that affect the colour mutation [26].

The coordinates of CIELAB colour space can predict the total colour variation among the plants by calculating the value of the total colour difference " $\Delta E^*$ ". The disparity is a numerical value of the colour alteration. The variation in  $\Delta E^*$  relies on the stress component, and the distinction between  $a^*$  and  $b^*$ . In order to quantify the stress, the evaluation of the  $\Delta E^*$  is required. For water stress conditions in this study,  $\Delta E^*$  was determined using the following formula [23]:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

here,  $\Delta L^*$ ,  $\Delta a^*$  and  $\Delta b^*$  indicates the difference between the colour parameters  $L^*$ ,  $a^*$  and  $b^*$  of day 1 and after 7, 13, 23, and 33 days for each plant. The first day of water stress was considered a reference and a higher  $\Delta E^*$  means the variation in colour is more compared to the reference. From Figure 5, it can be shown that the well-watered plant (plant 3) significantly delayed the increase in  $\Delta E^*$  compared to water-stressed plants (plants 1 and 2). In addition, the value of  $\Delta E^*$  increased rapidly with water stress time for plant 1. Furthermore, from Figure 5, the experimental data for the difference in the colour for plant 1 and plant 2 was fitted to zero-order ( $C = C_o \pm kt$ ) and first-order ( $C = C_o e^{\pm kt}$ ) degradation reaction kinetics respectively to describe the variation in colour [14]. From the calculation, the results showed that the model kinetic constant ( $k$ ) values were  $0.306 \pm 0.057$  ( $Day^{-1}$ ) and  $0.010 \pm 0.006$  ( $Day^{-1}$ ) with coefficient of determination " $R^2$ " of 0.935 and 0.999 for plants 1 and 2, respectively. From the value of the kinetic rate constant of the colour change, it can be deduced that the water stress enhanced the level of colour damage.



**Figure 5.** The total colour difference of oil palm seedlings during water stress time.

#### 4. Conclusion

In this study, the water stress of three oil palm seedlings (*Elaeis Guineensis*) was examined by evaluating the colour of leaves using the CIELAB colour system. Plants 1 and 2 were subjected to water-deficit stress and then to water and light stress, while plant 3 was daily watered and considered a reference. The seedlings were subjected to water stress for 33 days and then to both water and light stress from 55 day to 78 day. It was found that after 33 days of water stress, the values of  $L^*$  for plants 1, 2, and 3 were 37.19, 44.0, and 35.76, respectively. On the other hand, after water and light stress starting from day 55 to day 78, the values of  $L^*$  were 48.48, 53.23, and 34.80 for plants 1, 2, and 3, respectively. In addition, depending on  $L^*$ , the colour of the leaves of the seedlings under water stress and daily watered seedlings was clearly distinguished from each other. It was observed that there is a slight change in the greenness (negative  $a^*$ )/yellowness (positive  $b^*$ ) in plant 3 compared to other plants when they were subjected to water and light stress. This means that the water stress combined with light stress more affected the colour of the leaves compared to light stress alone. Understanding the water deficit stress in oil palm plants is of great importance and is also a fundamental part of making the crops stress realization.



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