Physiological character analysis of two shallot varieties at various watering frequencies

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Abstract. Analysis of the physiological characteristics of plants is important because the analysis includes various observations, calculations, as well as the varying relationships between plant growth and development processes and their results. The purpose of this study was to analyse the physiological characters of two shallot varieties at various watering frequencies. The research design used was a randomized block design with 2 factors, namely shallot varieties (Medan and Bima Brebes) and the frequency of watering (intervals of 1 once a day, once every 3 days, once every 5 days and every 7 days) located in the Greenhouse and Tissue Culture Laboratory of the Faculty of Agriculture, Universitas Sumatera Utara in May - September 2019. The results indicated that the Bima Brebes variety produced the highest total chlorophyll, which was significantly different from the Medan variety. The leaves relative water content was not significantly different in the two shallot varieties tested by watering frequency treatment. Watering once in 5 days produced the lowest hydrogen peroxide but produced the highest ascorbate peroxidase enzyme. The activity of the ascorbate peroxidase enzyme was significantly different and had the highest value in the Medan variety, watered with a frequency of once in 5 days.

1. Introduction
Drought conditions are a global obstacle for shallot production. When plants experience drought conditions, morphological or physiological adjustments will occur in response to drought stress. The adjustments made by plants to drought are highly dependent on the level of stress experienced, the phase of plant growth when experiencing stress, and the type or variety of plants. Drought is related to water availability which is an important factor in plant growth. Water availability is an important requirement for optimal bulb yield quality [1].

Efforts that can be made to deal with drought stress are watering. Watering is the process of applying water to the soil to meet plant needs. Providing adequate amounts of water at the right time is the goal of watering the plants to facilitate soil cultivation, regulate soil temperature, microclimate, clean or wash the soil from dissolved salt, and eradicate pests and diseases [2]. The appropriate watering intensity can support plant growth and development. Lack or excess of water in shallot planting medium can interfere with its production. Shallots are plants that require a lot of water but cannot withstand puddles or stagnant conditions [3].

Analysis of plant physiological characters is important because this analysis includes various observations, calculations, and varying relationships between plant growth and the yields. This means that with a good growth process, good plant results will be obtained. This is due to the support of various environmental factors suitable for the growing character. The function of observing
physiological characters is to determine the activity of enzymes such as superoxide dismutase (SOD), peroxide dismutase (POD) and ascorbate peroxidase (APX) enzymes [4]. The results of Ariska and Rachmawati’s research [1] showed that the differences in watering frequency and cultivars type affected the growth and yield of three shallot cultivars. The purpose of this study was to analyze changes in physiological characters of two shallot varieties at various watering frequencies.

2. Materials and methods
This research was conducted for five months, namely May - September 2019. Planting shallots and watering treatment were carried out in a greenhouse and analysis of physiological characters was carried out at the Tissue Culture Laboratory of Faculty of Agriculture Universitas Sumatera Utara. The research design used a randomized block design with two factors, namely shallot varieties (Medan and Bima Brebes) and watering frequency (intervals of once a day, once in 3 days, once in 5 days and once in 7 days). The observed Parameters were total chlorophyll (Wintermans and De Mots, 1965 method), leaf relative water content (Prochazkova et al. 2001 method), hydrogen peroxide content (Sergiev et al., 1997 method), and ascorbate peroxidase enzyme activity (Nakano and Asada, 1981 method). The observation of physiological parameters was carried out at 42 days after planting.

The research stages started from the preparation of planting medium, planting using polybags, watering according to treatment, plant maintenance, and taking leaf samples for physiological character analysis. Data processing was carried out by analyzing the variety (F test) to see the effect of the treatment and then continued with the DMRT (Duncan's Multiple Range Test) at the 5% level to see the differences between treatments.

3. Results and discussion
The ability of plants to adapt to drought stress depends on the intensity and period of stress, growth phase and plant genotype. Each plant variety has a very complex reaction in dealing with drought stress as indicated by changes in morphology and different plant physiology [5]. Measurement of physiological characters such as chlorophyll content is an approach to study the effect of water deficiency on growth and yield, because this parameter is closely related to the rate of photosynthesis [6]. The results in Table 1 indicated that the two varieties had significantly different total chlorophyll content. Chlorophyll in Bima Brebes variety was 21.69% higher than in Medan variety. Hendriyani et al [7] and Biber [8] stated that total chlorophyll content is related to various factors such as plant age, leaf age, morphology, genetic factors, water and oxygen availability and nutrients.

Table 1. Total chlorophyll content of two shallot varieties on various frequency of watering at 42 days after planting (DAP).

| Variety  | Watering Frequency | Mean            |
|----------|--------------------|-----------------|
|          | Once a day         | Once in 3 days  | Once in 5 days | Once in 7 days |
| Medan    | 1.460              | 1.584           | 1.313          | 1.360          | 1.429 b       |
| Bima Brebes | 1.896              | 1.325           | 1.892          | 1.844          | 1.739 a       |
| Mean     | 1.678              | 1.455           | 1.602          | 1.602          |

Note: Different superscript letters on the interaction matrix, the mean of column showed significant differences based on Duncan's multiple distance test (p <0.05)

The frequency of watering does not affect the total chlorophyll content of shallots (table 1). Plants watered every day had the highest total chlorophyll content compared to other treatments. Water deficit will affect the chlorophyll content and organization in the chloroplasts tissue. According to Hendriyanti and Setiani [9], low water content causes the transportation of ammonium to be less than optimal. In addition, the low water content in the planting medium will directly inhibit chlorophyll synthesis in leaves. The lack of water availability causes the photosynthetic rate to decrease which
results in decreased chlorophyll synthesis. Lack of water also causes an increase in temperature and transpiration, causing disintegration of chlorophyll. The results of Arifina et al [10] research also stated that sweet potato plants which applied with adequate water during their growth period had higher total chlorophyll content than plants with limited amounts of water.

Table 2. Leaves relative water content of two shallot varieties on various frequency of watering at 42 DAP.

| Variety     | Watering Frequency | Mean     |
|-------------|--------------------|----------|
|             | Once a day         | %        |
|             | Once in 3 days     |          |
|             | Once in 5 days     |          |
|             | Once in 7 days     |          |
| Medan       | 37.154             | 34.831   |
|             |                    | 41.167   |
|             |                    | 35.304   |
|             | 37.114             |          |
| Bima Brebes | 34.981             | 29.659   |
|             |                    | 35.819   |
|             |                    | 27.195   |
|             | 31.914             |          |
| Mean        | 36.069             | 32.245   |
|             |                    | 38.493   |
|             |                    | 31.250   |

The data in table 2 showed that the relative water content of the two shallot varieties tested and the watering frequency treatment was not significantly different. The leaves relative water content in Medan variety was higher than Brebes variety. This indicated that Medan shallot variety has better adaptability to environmental as indicated by its ability to maintain the relative water content of the leaves by developing a more extensive root system than the Bima Brebes variety. The relative water content of leaves is a variable of plant resistance to drought stress [11], because it describes the water status and turgor pressure of leaf cells, especially when the plant has decreased water potential. Research by Dewi et al. [12] showed that the drought tolerant genotype of millet had better leaf relative water content than sensitive genotype.

The shallot plants that were watered once in 7 days had the lowest leaf relative water content compared to other treatments. This data indicated that plants with the longest watering frequency experienced drought stress which resulted in low leaf relative water content. Research by Dewi et al. [12] also showed a similar result trend, in which the relative water content of millet leaves decreased with lower field capacity content in the growing medium. Some researchers explained that the relative water content of leaves is a variable of plant resistance to drought stress, because it describes the water status and turgor pressure of leaf cells, especially when the plant has decreased water potential. Drought stress causes the turgor pressure to drop, so that the stomata closes. A decrease in the relative water content of the leaves will reduce the stomata conductance of the leaves and will slowly decrease the concentration of CO\textsubscript{2} in the leaves so that it can reduce the rate of photosynthesis [11, 13].

The results in Table 3 showed that hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) had no significant effect on the two shallot varieties at 42 days after planting. The hydrogen peroxide content of Medan variety was 25% higher than Bima Brebes variety, which was more tolerant of drought stress. The two shallot varieties tested produce toxic free radicals that can interfere with the metabolic processes in plants. The same trend was also shown by the research of Chakraborty and Pradhan [14] which showed that drought stress resulted in less H\textsubscript{2}O\textsubscript{2} accumulation in tolerant wheat varieties than sensitive wheat varieties. Foyor and Noctor [15] explained that drought stress will cause plants to activate a defense system in the form of Reactive Oxygen Species (ROS), but if the ROS levels are excess it will cause oxidative damage. ROS are free radicals that reactively bind to other molecules in cells. One type of ROS plays an important role as a signaling molecule for various plant physiological processes, namely hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}). Hydrogen peroxide also plays an important role in the signal transduction process for plants that are tolerant of abiotic stress.

The treatment of watering frequency had a significant effect on the hydrogen peroxide content produced by shallot plants. Plants watered every day produce the highest hydrogen peroxide and were significantly different from plants watered once in 5 days. This is presumably because plants which were watered once a day causing the planting medium becomes too moist and the plants experience
excess water stress, resulting in higher hydrogen peroxide. Watering once in 5 days is the right frequency to meet the water needs of the shallot plant. Research by [13] also showed an increase in the hydrogen peroxide content of *Sesamum indicum* treated with the inundation. Furthermore, Hasanuzzaman and Fujita [14] stated that this excess amount of H$_2$O$_2$ must be considered because these free radicals easily penetrate the membrane and are not compartmented in the cell. H$_2$O$_2$ can also deactivate an enzyme by oxidizing its thiol groups.

### Table 3. Hydrogen peroxide content of two shallot varieties on various watering frequency at 42 DAP.

| Variety      | Watering Frequency | Mean     |
|--------------|--------------------|----------|
|              | Once a day         | Once in 3 days | Once in 5 days | Once in 7 days |
| Medan        | 0.226              | 0.113     | 0.062         | 0.120         | 0.130         |
| Bima Brebes  | 0.105              | 0.110     | 0.081         | 0.120         | 0.104         |
| Mean         | 0.166$^{a}$        | 0.111$^{ab}$ | 0.071$^{b}$  | 0.120$^{ab}$  | 0.125         |

Note: Different superscript letters on the interaction matrix, the mean of row showed significant differences based on Duncan's multiple distance test ($p < 0.05$)

The data listed in table 4 shows that the frequency of watering significantly affects the activity of the ascorbate peroxidase (APX) enzyme in the two shallot varieties studied. In line with the amount of hydrogen peroxide produced, the Medan variety also showed higher ascorbate peroxidase enzyme activity than the Bima Brebes variety. The trend of increasing APX enzyme activity in soybean plants studied by Violita and Hamim [16] was directly proportional to the increase in drought stress treatment given, especially in soybean varieties that produced more H$_2$O$_2$. Oxidative stress in plants can induce the plant to form a defense system by means of a rescue mechanism. This rescue mechanism, among others, is through changes in the activity of antioxidant enzymes, including ascorbate peroxidase (APX). Caverzan et al [17] described that in plant cells, the main detoxification system for hydrogen peroxide is through the ascorbate-glutathione cycle. In this system, the ascorbate peroxidase enzyme plays a very important role in the catalysis process that converts H$_2$O$_2$ into water by using ascorbate as a specific electron donor. This condition causes plants to avoid environmental stress.

### Table 4. Ascorbate peroxidase enzyme activity of two shallot varieties on various watering frequency at 42 DAP.

| Variety       | Watering Frequency | Mean     |
|---------------|--------------------|----------|
|               | Once a day         | Once in 3 days | Once in 5 days | Once in 7 days |
| Medan         | 0.117$^{b}$        | 0.083$^{b}$  | 0.656$^{a}$   | 0.243$^{b}$   | 0.276         |
| Bima Brebes   | 0.113$^{b}$        | 0.231$^{b}$  | 0.172$^{b}$   | 0.201$^{b}$   | 0.126         |
| Mean          | 0.125              | 0.161     | 0.414         | 0.225         |

Note: Different superscript letters on the interaction matrix, the mean of column and row showed significant differences based on Duncan's multiple distance test ($p < 0.05$)

The results showed that the highest APX enzyme activity was found in the Medan variety which was watered once in 5 days, which was significantly different from other treatment combinations. This indicated that there was a balance of APX activity in the treatment combination hence metabolic disorders in cells caused by free radicals can be minimized. Lin and Wang [18] stated that the ability of antioxidant enzymes in dealing with oxidative stress varies. Therefore the balance between ROS...
and antioxidant ability is very important for plants. In addition, each plant genotype has a different balance between ROS and antioxidants therefore each genotype has a different resistance.

4. Conclusions
The highest amount of total chlorophyll was produced in the Bima Brebes variety, which was significantly different from the Medan variety. The two varieties of shallots and the watering treatment had no significantly different on leaf relative water content. The hydrogen peroxide produced by the two shallot varieties was not significantly different, while the treatment of watering frequency was significantly different in this parameter with the highest hydrogen peroxide content at the frequency of watering once a day. The interaction between Medan varieties and the frequency of watering once in 5 days resulted in the highest ascorbate peroxidase enzyme activity and was significantly different from other treatment combinations.

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