Physiological Characteristics Analysis of Leaves of Several Sweet Potato (*Ipomoea batatas* L.) Genotypes on Various Watering Level

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Abstract: Drought stress is a major problem in crop production worldwide. The unavailability of groundwater and erratic climate change causes a lack of water for plants. Efforts that can be made to maintain sweet potato production in drought conditions are by planting genotypes that are tolerant of drought stress. This research aim was to determine the growth and production and also to analyze the physiological characters of several sweet potatoes genotypes on various watering level. The observed parameters were chlorophyll a, b and total and also relative water content at two months and three months after planting (MAP). The results showed that sweet potato genotypes had a different response to the level of watering. The response of each sweet potato genotype was significantly different in the parameters of chlorophyll a, b and total chlorophyll in the third month and the relative water content at 2 MAP. The Binjai accession genotype had the highest chlorophyll a, b and total chlorophyll content compared to other genotypes at 3 MAP. Watering levels treatment significantly affected the chlorophyll a, b and total at 3 MAP. The optimum watering significantly increases the chlorophyll a, b and total chlorophyll content at 3 MAP.

1 INTRODUCTION

Drought stress is an environmental condition in which plants do not receive sufficient water intake hence plants cannot carry out the process of growth and development optimally. Drought stress is a major problem in crop production worldwide. Drought stress is identical to lack of water hence if plants experience a lack of water, the stomata in the leaves will close and will result in CO_2 being blocked to enter and reduce photosynthetic activity in the plant. In addition, plants will also experience inhibition in synthesizing proteins and cell walls (Farooq *et al*., 2009).

Water requirements of each plant are different, depending on the type of plant and its growth phase. Water requirements in plants can be fulfilled with the absorption of water by the roots. The amount of water absorbed by roots is very dependent on soil water content, the ability of soil particles to hold water and the ability of roots to absorb water (Song *et al*., 2010). Plants that experience a lack of water generally have a smaller size compared to plants that grow normally. Water shortages can reduce crop yields very significantly and even can cause death in plants (Song and Banyo, 2011).

Sweet potatoes are said to be (1) tolerant of drought: if the decrease in tuber yield is less than 10% against normal watering, (2) moderate tolerance: if the decrease in tuber yield ranges from 11-20% to normal watering, (3) sensitive: decreased in tuber yields range from 21-40%, and (4) very sensitive: a decrease in yield is >40% compared to normal watering (Chunsheng *et al*., 1993).

Lizhen (1995) researched two sweet potato varieties and reported that the critical phase of sweet potato plants on drought was at the beginning of growth, i.e. at 1-60 days after planting (DAP). In general, the phase of tubers formation in sweet potato ranges from 30–45 DAP. In this phase, if there is a drought, it will reduce canopy weight, leaf area, and tuber yield.

Varieties are one of the important technological components that are easily adopted by farmers. The planting of sweet potatoes which were intended for consumption is preferred with varieties that taste sweet, form a good tuber, and have a low water content (Yusnita, 2010). Beta-1 is a sweet potato variety which has a high beta-carotene content, exceeding the beta-carotene content of 12,032 µg/100gram this is even higher than beta-carotene levels in carrots. The high content of beta-carotene

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can be predicted from the color of orange flesh. The yield potential of this variety reaches 35.7 tons/ha with a harvest age of 4.0-4.5 months. The advantages of Beta-1 sweet potato varieties have high production potential and beta-carotene content (Jusuf *et al*., 2008).

With the availability of superior varieties that have good tolerance to drought, sweet potatoes can be commercially managed and yield losses and production costs can be reduced. Sweet potato plant breeding which aimed at improving tolerance to drought has not been specifically done in Indonesia (Jusuf *et al*., 2005).

Zulkadifta (2018) research showed that the local sweet potato genotype from Lubuk Pakam resulted in the number of tubers, tuber length, tuber weight and harvest index. This was presumably because, in the local genotype of Lubuk Pakam, the photosynthesis results were mostly translocated to tubers hence the production was greater than that of other genotypes, local genotypes of Lubuk Pakam and Perbaungan were able to adapt in planting environment while the Beta 1 genotype from Malang had to adapt first in planting environment. Local sweet potato genotype of Perbaungan has higher tuber weight and harvest index because local genotypes are able to adapt, grow and produce better than introduced varieties.

Increasing production of sweet potato plants can be done through fertilization and watering. Availability of water is a limiting factor for plant growth. To fulfill the water needs of the plant and maintain its availability in the soil and its distribution, watering is needed. Another component in increasing production is watering which is an essential factor for plants (Sari *et al*., 2016).

The soil moisture required by sweet potato plants at the beginning of growth ranges from 60-70%, in the middle of the growth is 70-80%, and the end of growth requires 60% humidity. However, long drought duration can inhibit tuber growth hence it can affect the results (Flach and Rumawas, 1996). The critical phase of sweet potato in the condition of water deficit is at the beginning of growth (1-60 DAP). Decrease in canopy weight, leaf area and tuber yield can occur in these conditions. Loss of fresh tuber yield due to drought stress is reported range from 2.53 to 63.52%. The amount of tuber yield loss depends on the intensity of stresses, the type of soil and varieties/clones used (Lizhen, 1995).

Rahayuningsih *et al*., (2000) evaluated 50 sweet potato clones against drought by normal watering from planting to harvesting and limited watering treatment until 1.5 months after planting (MAP). The results showed that the range of tuber loss was between 2.53-63.52% in conditions of drought.

Irrigation in sweet potato plants on drought consisting of three levels of irrigation, namely: P0 (very limited irrigation) = plants were irrigated from planting to four weeks after planting with an interval of 10 days, P1 (limited irrigation) = plants were irrigated from planting to eight weeks after planting with an interval of 10 days, P2 (optimum irrigation) = plants were irrigated from planting to harvest with an interval of 10 days (*Hapsari et al*., 2011). Drought in sweet potatoes can cause a decrease in tendrils length by 25% (60 hst) and 29% (90 hst). The impact of drought also causes leaf area to decrease by 30% and tuber weight in very large categories (> 300g) decreases by 33% (Hapsari and Mejaya, 2016).

This research aim was to determine the growth and production and also analyze the physiological characteristics of several sweet potatoes (*Ipomoea batatas* L.) genotypes on various levels of watering.

2 MATERIALS AND METHODS

2.1 Research Area

The experiment was conducted in April-December 2018 at the experimental field of the Faculty of Agriculture, Universitas Sumatera Utara, Prof. A. Sofyan Street, No. 3 Kampus USU, Medan. This research used a randomized block design with two factors, the first factor was sweet potato genotype (Beta-1 superior variety, Perbaungan local accession, and Binjai local accession) and the second factor was watering levels, very limited watering (watered until 1 month with 10 days interval), limited watering (watered until 2 months with 10 days interval) and optimum watering (watered until 4 months with 10 days interval). This research started from land preparation, planting, application of watering, tending, fertilizing, and physiological parameter analysis which includes the content of chlorophyll a, b, and total and the relative water content of leaves at Laboratorium Kultur Jaringan Tanaman, Faculty of Agriculture, University of Sumatera Utara. Observations were made in the second and third months after planting. Watering applications were carried out starting 4 weeks after planting (WAP) up to 9 MST with 10 days intervals.

2.2 Analysis Procedures of Chlorophyll a, b and Total Content

The chlorophyll analysis method used in calculating the amount of chlorophyll a, b and total was the Wintermans and De Mots method (1965). Chlorophyll was extracted by crushing the leaves using 96% ethanol. After that filtered using filter paper, then the solution was transferred into a test tube hence 25 ml of leaf extract was obtained. Prepared UV/VIS spectrophotometer and arranged the wavelength, inserted 96% ethanol solution (blank) as neutralizer then released the blank solution then alternately put the extract solution into the UV/VIS spectrophotometer. The solution was measured by a spectrophotometer at wavelengths of 649 nm and 665 nm. Total chlorophyll, chlorophyll a, chlorophyll b in units of g/mg were calculated using the formula:

$$\text{chlorophyll a} = \{(13.7 \times A665) – (5.76 \times A649)\}/10 \quad (1)$$
$$\text{chlorophyll b} = \{(25.8 \times A649) – (7.60 \times A665)\}/10 \quad (2)$$
$$\text{Total chlorophyll} = \{(6.10 \times A665) + (20.0 \times A649)\}/10 \quad (3)$$

A665 = absorbance of chlorophyll extract at 665 nm
A649 = absorbance of chlorophyll extract at 649 nm
Analysis of chlorophyll content was carried out when the plants were 2 months and 3 months old.

2.3 Leaf Relative Water Content (RWC)

The relative water content of leaves was analyzed using *Prochazkova et. al*., (2001) method. Relative water content was determined by taking 10 pieces of leaves. The leaf pieces were weighed using an analytical scale to find out the fresh weight (FW). Then, hydration was done for 24 hours. After 24 hours weighing was carried out to determine saturated weight (SW). To find out the dry weight (DW), the leaf pieces were oven-dried at 80^0C for 48 hours. The activity of relative water content is expressed in units of percent. Relative water content was calculated by the formula:

$$\text{RWC} = \frac{\text{Fresh weight - Dry weight}}{\text{Saturated weight - Dry weight}} \times 100\% \quad (4)$$

2.4 Data analysis

Data were analyzed statistically by the F test and continued by the Duncan Multiple Range Test (DMRT) at the level of α 5%.

3 RESULTS AND DISCUSSIONS

3.1 Leaf Chlorophyll Content

The data presented in Table 1 showed that the three genotypes were not significantly different from the parameters of chlorophyll a, b and total chlorophyll at 2 MAP. Beta-1 varieties had the highest chlorophyll a and total chlorophyll content compared to other genotypes, while the highest chlorophyll b content was produced by the Binjai accession genotype. It was suspected that at the beginning of planting the water content in the rhizosphere was still sufficient to process the growth of the three sweet potato genotypes, including the formation of chlorophyll. Chlorophyll content is influenced by genetic factors, plant growth rates, adaptability of plants and the environment. Factors that influence the formation of chlorophyll include genes, light, and elements of N, Mg, Fe as forming and catalyst in the synthesis of chlorophyll. All green plants contain chlorophyll a and chlorophyll b. Chlorophyll a composes 75% of total chlorophyll. Chlorophyll content in plants is about 1% dry weight (Pratama and Laily, 2015). The formation of chlorophyll in leaves is most influenced by sunlight, but the age of the leaves also affects the chlorophyll content found in a leaf (Hidayat, 2008).

Table 1: The content of chlorophyll a, b and total of several sweet potato genotypes at 2 MAP.

Genotypes	chlorophyll a (mg/g)	chlorophyll b (mg/g)	Total chlorophyll (mg/g)
Beta-1 Variety	50,79	28,57	25,36
Perbaungan accession	43,82	29,08	22,27
Binjai accession	28,34	34,51	15,78

Plants that are able to adapt in one environment will have a higher chlorophyll content than plants that are unable to adapt. The data presented in Table 2 showed that the chlorophyll content was significantly different in each genotype at 3 MAP. The Binjai accession genotype had the highest chlorophyll a, b and total content compared to other genotypes. This was because the Binjai local accession genotype has good adaptability even though it was planted in a different environment from its origin. Jusuf *et al*., (2008) stated that varieties/clones/genotypes that are broadly adapted have the advantage of being able to produce high yields on diverse agroecosystems. Chipungu *et. al*., (2018) also stated that environmental factors such as soil type, soil pH, altitude, planting season, and temperature greatly influenced the tuber yield when compared with genotype and genotypic and environmental interactions.

Table 2: Chlorophyll a, b and total content of several sweet potato genotypes at 3 MAP.

Genotypes	chlorophyll a (mg/g)	chlorophyll b (mg/g)	Total chlorophyll (mg/g)
Beta-1 Variety	13,74c	12,59c	7,29c
Perbaungan Accession	17,83b	15,91b	9,42b
Binjai Accession	23,18a	20,82a	12,26a

Description: The numbers followed by the different letters in the rows in each average show significantly different based on Duncan's Multiple Range Test at the level of α = 5%.

The data presented in Table 3 showed that the watering level treatments did not significantly affect the content of chlorophyll a, b and total chlorophyll at 2 MAP. The need for water for the formation of chlorophyll is still sufficient for plants hence the process of chlorophyll formation had not been disturbed. Research by Hendriani and Setiari (2009) also showed that the content of chlorophyll a, b and total in long bean leaves (*Vigna sinensis*) is not significantly different at various levels of watering.

Table 3: The effect of several watering level treatment on chlorophyll a, b and total content at 2 MAP.

Watering Levels	chlorophyll a (mg/g)	chlorophyll b (mg/g)	Total chlorophyll (mg/g)
Very Limited Watering	49,18	30,60	24,81
Limited Watering	39,55	30,42	20,47
Optimum Watering	34,22	31,14	18,13

The data presented in Table 4 showed that the watering levels had a significant effect on chlorophyll a, b and total content at 3 MAP. The optimum watering level had the highest chlorophyll a, b and total content compared to other treatments. In the optimum watering treatment, which was watered until 4 months old with 10 days interval, the planting medium had sufficient water content to meet the water needs of plants to grow well and perform various metabolic activities such as photosynthesis. Rahayuningsih *et al*. (2000) stated that the chlorophyll content tends to decrease in line with the length of the time interval for watering. Fitter and Hay (1991) also stated that lacking or excessive water condition resulting in disruption of plant physiological process, or can cause plants to become stressed and if it lasts for a long time, plants will experience wilting even die.

Table 4: The effect of several watering levels on chlorophyll a, b and total content at 3 MAP.

Watering levels	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)
Very Limited Watering	15,04c	13,25c	7,93c
Limited Watering	18,66b	16,70b	9,86b
Optimum Watering	21,05a	19,37a	11,17a

Description: The numbers followed by the different letters in the rows in each average show significantly different based on Duncan's Multiple Range Test at the level of α = 5%.

3.2 Leaf Relative Water Content (RWC)

The data presented in Table 5 showed that the leaf relative water content was significantly different in the three sweet potato genotypes at 2 MAP. The Binjai accession genotype had the highest relative water content of 42.66% compared to other genotypes. It was suspected that the Binjai accession genotype had a better ability to adapt in its growth environment than other genotypes hence it can develop a better root system to absorb water and maintain the leaf relative water content.

Whereas three months after planting, the relative water content of the leaves in the three genotypes was not significantly different. It was suspected that the three genotypes had similar adaptive abilities in line with the increasing age of the plants. The research results of Khalili *et al*. (2011) reported that relative water content is affected by season and irrigation hence drought stress can reduce the relative water content significantly. Plants that have a relative water content between 18.6 - 21.8%. Furthermore, it was said that the difference in relative water content ranging between 18.6% and 21.8% is the plant genotype which is most resistant to drought stress. Fitri and Salam (2017) also stated that in sufficient water conditions the development of roots will be perfect and can absorb available nutrients hence it can increase plant growth, but if there is a lack of water, the growth will be hampered especially in the vegetative phase. The existence of sufficient water during plant growth resulted in the process of nutrients absorption and the rate of photosynthesis going smoothly hence it can increase plant growth.

Table 5. Leaf relative water content of several sweet potato genotypes at 2 and 3 MAP.

Genotypes	Relative Water Content (%)	
	Two Months After Planting	Three Months After Planting
Beta-1 Variety	29,47c	44,48
Perbaungan Accession	38,13b	42,17
Binjai Accession	42,66a	42,99

Description: The numbers followed by the different letters in the rows in each average show significantly different based on Duncan's Multiple Range Test at the level of α = 5%.

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Table 6: The effect of several watering levels on the leaf relative water content at 2 and 3 MAP.

Watering Levels	Relative Water Content (%)	
	2 months after planting	3 months after planting
Very Limited Watering	39,22	46,01
Limited Watering	35,87	42,78
Optimum Watering	35,17	40,86

Table 6 showed that the watering levels had no significant effect on the parameters of leaf relative water content at 2 and 3 MAP. Plants with very limited watering treatment had a high leaf relative water content compared to other watering levels. This was because the value of relative water content is inversely proportional to the potential of leaf water, where in plants that get drought stress, namely at very limited watering levels, will experience more severe stress due to the amount of water used by plants to maintain osmotic pressure and transpiration are greater hence the water potential decreases. Correspondingly, Makbul *et al*. (2011) reported that the response of plants to drought is very complex, including several changes as a step of adaptation. Furthermore, it is stated, drought stress is the status of water in plants that can be known by measuring the potential of leaf water and relative water content as physiological indicators. Water status on leaves, usually an interaction between leaf water potential and stomatal conductance, in which drought will induce root signals to the canopy to reduce the rate of transpiration hence the stomata closes when the water supply decreases. High relative water content is a mechanism of plant resistance to drought, and this is the result of excessive osmotic regulation or a reduction in the elasticity of cell wall tissue.

4 CONCLUSIONS

The three sweet potato genotypes had chlorophyll a, b and total contents which were significantly different when the plants were at three months after planting. While the leaf relative water content in the three genotypes was significantly different at two months after planting. Binjai accession sweet potato genotype had the highest chlorophyll a, b and total chlorophyll content compared to other genotypes at 3 MAP. The treatment of watering levels significantly affected the chlorophyll a, b and total content at 3 MAP. Watered plants with optimum watering levels had the highest chlorophyll a, b and total content at 3 MAP, while plants with very limited watering treatment had the highest leaf relative water content at 2 and 3 MAP.

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