The Effect of leaf surface character on the ability of water hyacinth, *Eichhornia crassipes* (Mart.) Solms. to transpire water

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Abstract. The objectives of this study were to evaluate the effect of leaf surface character on the ability of water hyacinth (*Eichhornia crassipes* (Mart.) Solms.) to transpire water. This experiment was performed using a healthy and acclimatized water hyacinth collected from Sawangan, Depok area. Five individual plants with the same size (6 leaves per plant), leaf length (10.4 ± 0.8 cm), leaf width (7.8 ± 0.7 cm) and root length (24.8 ± 7.4 cm) were put in each 20 L plastic tank containing tap water and allowed to grow in a greenhouse for six days. The transpiration rate of each plant was measured using a weighing method every day. The number of stomata, stomatal density and stomatal index were measured; amount of water loss from the individual plant through transpiration was analyzed. Based on the results of the study, it was found that the lowest leaf surface area is correlated with the number of stomatal and transpiration rates in the individual measured plants.

Keywords: *Eichhornia crassipes*, transpiration, number of stomata, stomata index

1. Introduction

Plants absorb water to fulfill their metabolic needs. Water absorption is influenced by several factors, one of which is the transpiration rate where the higher the transpiration rate, the higher amount of water will be absorbed by the roots [1]. Transpiration is the loss of water from the body of plants in the form of steam through stomata, cuticles or lenticels [2]. Approximately 90% of the water contained in plants is transmitted out through the stomata. This is due to the breadth of the leaf surface and also the leaf surface which is more exposed to sunlight than other parts of a plant. In most plant species, transpiration via the cuticle is only 10% [3].

The process of transpiration is influenced by various factors, namely internal and external factors. Internal factors include leaf size, the thickness of leaves, the presence or absence of a wax layer on the leaf surface, the number of trichome on the leaf surface, the number of stomata, and the shape and location of the stomata. External factors include the light intensity, temperature, air humidity, wind speed and groundwater content, potential groundwater gradients-tissue-atmosphere, and the presence of toxic substances in the environment [4]. Transpiration plays a role in the process of transporting water to the leaves and water diffusion between cells, absorption, and transport of water and nutrients, transport of assimilates, removing excess water, regulating stomata openings, and maintaining leaf temperature. Transpiration is closely related to stomata which are an internal factor that has the most influence on the process of transpiration rate. The greater the number of stomata, the higher the rate of...
transportation [5]. The number of water hyacinth stomata on the upper surface of the leaf is more than the bottom surface of the leaf. This is one form of adaptation of aquatic plants to accelerate the evaporation process so that physiological temperatures in plants are better maintained [4].

Water hyacinth has the ability to breed very quickly around 3%/day so that the water hyacinth is able to cover the entire surface of the water [6]. Water hyacinth can survive in waters for six years and has a high ability to grow in highly polluted water to absorb nutrients, metal ions, and organic pollutants. Besides the ability to absorb heavy metals, water hyacinth has some adverse effects including increase sedimentation and evapotranspiration. The loss of water by water hyacinth can reach three times greater than the rate of natural evaporation of surface water that does not have water hyacinth [7]. Studies have been conducted, evaporation of open water averaging 4.3 mm per day and evapotranspiration of aquatic plants has an average of 7.8 mm per day [8]. The increase of water loss through evapotranspiration of water hyacinth is considered as one of the most important weed problems in water bodies. Therefore, it is necessary to conduct a research about the leaf surface character on the ability of water hyacinth (*Eichhornia crassipes* (Mart.) Solms.) to transpire water. So, it can be estimated how long the water hyacinth can be used effectively without causing sedimentation.

2. Materials and method

2.1. Plant materials

The study was conducted in the greenhouse of FMIPA UI in June–July 2019. Water hyacinth were collected from Sawangan area, Depok. Five individual plants with the same number of leaves, leaf width, and stem height were selected for the experiments. After the selection, they were rinsed with tap water and placed in a 20 L plastic tank filled with tap water for 8 days to let them adapt to the new environment.

2.2. Measurement of transpiration

The rate of transpiration can be investigated by measuring the decrease in mass due to water loss. The experiment was done by weighing method conducted in the greenhouse of FMIPA UI. Each of five plants was placed individually in a plastic tank filled with 6 L of tap water. A thin film of oil was added to the surface of the water to prevent loss of water by evaporation. After that, the initial weight was calculated using a digital weighing scale (50 kg capacity). The transpiration was carried out for 6 days. During the experiments, temperature, wind speed, humidity, and light intensity were recorded at the exact time i.e. 11.00–12.00 a.m. On the 6th day, the final weight of the water hyacinth was measured to determine the amount of water loss from every tank. Any difference in weight will indicate the loss of water by the plant (due to transpiration). The transpiration rate is measured as the amount of water lost expressed in units of g/cm²/h. After completing the transpiration process, each individual leaf was laid on a 1-mm grid paper and trace the outlines. All the leaf patterns were cut and the weight of the leaf patterns was measured using analytical scales.

2.3. Measurement of the number of stomata, stomatal density, and stomatal index

Each water hyacinth leaf was cut into three pieces and checked for the number of stomata both on the top and bottom of the leaf. Checking the amount of stomata was done by slashing the bottom and top layers of the water hyacinth leave that have been cleaned with alcohol before. After that the incision was transferred to the glass object that has been dripped with distilled water, then, one drop of safranin was added and covered with a glass cover [4]. The preparations were then examined using a Leica bl 500 microscopes with a magnification of 40 x 10. After that, a scale was given and numbered stomata and epidermal cells were used using ImageJ. The field of view of the microscope is 0.0798 mm² at a magnification of 40 x 10 with a scale of 50 µm. Stomata density can be calculated with the following formula [9]:

\[
\text{Stomata density} = \frac{\text{Total number of stomata}}{\text{Total area of leaf}}
\]
Stomatal density = \frac{\text{Number of stomata}}{\text{Field of view}}

Stomatal Index formula:

\text{Stomata Index} = \frac{\text{Number of stomatal}}{\text{Number of stomatal + number of epidermis cell}} \times 100 \%

3. Results and discussion

Based on table 1, it is known that the average number of stomata in the adaxial is higher than in the abaxial section. The number of stomata is correlated to the density of stomata, higher number of stomata, higher density of stomata. The stomata index is a comparison of the number of epidermal cells in a certain unit of area. The highest stomata index was shown in the 5th test with the lowest number of epidermal cells, while the lowest stomatal index was shown in the 2nd test with the highest number of epidermal cells. The highest transpiration rate was shown on the 3rd test and the lowest was on the 4th test. At the lowest transpiration rate, it is correlated to the number of stomata, stomata density, weight difference and lowest leaf area.

Based on the research that has been done, the obtained results of external factors, internal factors, number of stomata/field of view, number of epidermal cells/field of view, stomata index/field of view and density of stomata/field of view can influence the transpiration of *Eichhornia crassipes* plants. The process of transpiration is influenced by various factors, namely internal factors, and external factors. Internal factors include leaf area, the thickness of leaves, the presence or absence of a waxy layer on the leaf surface, the number of trichome on the leaf surface, the number of stomata, and the shape and location of the stomata.

Based on the study of the number of stomata on adaxial (9.6 ± 1.1) is more dominant than abaxial (9.4 ± 0.5) of the leaves, leaf area 327.91–468.93 cm², root length (24.8 ± 7.4 cm), leaf thickness of 0.028–0.034 μm, shape of stomata is paracytic, size of the upper surface stomata is 42.5 x 39.6 μm² and size of the lower surface stomata is 39.70 x 37.5 μm², have waxy layer and do not have trichome. The results of this study were consistent with other studies which stated that aquatic plants have a way to adapt to their environment such as large leaf area 532.96 cm², roots that are not too long 22.46 cm.

| Replication | Leaf surface | Number of stomata/field of view | Number of epidermal cell/field of view | Stomatal index (%)/field of view | Stomatal density/field of view | W0-W1 (kg) | Transpiration rate (g/cm²/hour) | Leaf area (cm²) |
|-------------|--------------|---------------------------------|---------------------------------------|---------------------------------|-------------------------------|------------|-------------------------------|----------------|
| 1           | Adaxial      | 10                              | 81                                    | 10.98                           | 125.31                        | 0.575      | 0.0204                        | 468.93         |
|             | Abaxial      | 9                               | 80                                    | 10.11                           | 112.78                        |            |                               |                |
| 2           | Abaxial      | 9                               | 84                                    | 9.67                            | 112.78                        |            |                               | 378.91         |
|             | Adaxial      | 10                              | 85                                    | 10.52                           | 125.31                        | 0.485      | 0.0201                        |                |
| 3           | Adaxial      | 11                              | 73                                    | 13.09                           | 137.84                        | 0.655      | 0.0275                        | 399.87         |
|             | Abaxial      | 10                              | 79                                    | 11.23                           | 125.31                        |            |                               |                |
| 4           | Adaxial      | 8                               | 60                                    | 11.76                           | 100.25                        | 0.39       | 0.0173                        | 327.21         |
|             | Abaxial      | 9                               | 71                                    | 11.25                           | 112.78                        |            |                               |                |
| 5           | Adaxial      | 10                              | 59                                    | 14.49                           | 125.31                        | 0.705      | 0.035                         | 394.95         |
|             | Abaxial      | 9                               | 66                                    | 12                               | 112.78                        |            |                               |                |
have a waxy layer, and stomata are generally large 47.5 μm x 35 μm and the location of the stomata is higher above the surface of the leaf [4, 10].

External factors include light intensity, temperature, air humidity, and wind speed [4]. Based on study, the average temperature is 29.41 °C, air humidity 49 %, wind speed 0–1.5 m/s and light intensity 974 Lux. The growth of water hyacinth in this study has met the optimal requirements. The fastest growth of water hyacinth is at 240,000 lux, and its minimum requirement is 24,000 lux, but it can grow under a broad range of light intensities, relative humidity of 15–75 %, a pH between 4.5–7.5, temperatures between 20–30 °C [11, 12].

3.1. Internal factors that affect transpiration rates

The leaf area influences the rate of transpiration. The wider the leaf area, the greater the rate of transpiration because wide leaves tend to have more stomata [5]. Figure 1 shows that the highest transpiration rate is shown in the 5th test with a value of 0.035, leaf surface area 394.5 cm² and the number of stomata 19. The lowest transpiration rate is shown on the 4th test with a value of 0.0173, leaf area 327.21 cm² and the number of stomata 17. Correlation analysis (figure 1) shows a positive relationship between the leaf area to the number of stomata with the correlation coefficient value of 0.505303. This shows that the correlation coefficient is moderate. The R square value obtained is 0.2553. These results indicate that the rate of transpiration which influenced by the number of stomata of 25.53 %. While the remaining 74.47 % is influenced by other factors.

The number and size of stomata are influenced by genotype and environment factors. The level of stomata density is influenced by the environmental factors such as: water availability, light intensity, temperature, and CO₂ concentration. The higher the intensity of light, the frequency of stomata on both leaf surfaces will be also increased, although the increase in frequency is not significant. Plants that grow in dry environments with high light intensity tend to have a lot of stomata, but their size is small compared to plants that grow in wet and protected environments. If the stomata are too close, it will inhibit the evaporation. Maximum water discharge occurs if the distance between stomata is 20 times its diameter. If the holes are too close together, then evaporation from one hole will inhibit the evaporation of the nearby holes. This is because the path taken by the water molecules passing through the hole is not straight but turn aside due to the influence of the corners of the closing cells [13].

Transpiration is closely related to stomata. Nearly 97 % of water from plants is lost through stomatal transpiration, so the more stomata, the higher the transpiration rate [3]. Correlation analysis (figure 2) regarding the relationship of the number of stomata to the rate of transpiration shows a positive relationship with the correlation coefficient value of 0.502162. This shows that the correlation coefficient is moderate. The R square value obtained is 0.2522. These results indicate that the rate of transpiration is influenced by the number of stomata of 25.22 %. While the remaining 74.78 % is influenced by other external and internal factors.

![Figure 1. Linear regression graph between leaf surface area and number of stomata.](image-url)
Figure 2. Linear regression graph between the number of stomata/field of view with the rate of transpiration.

$$y = 0.0026x - 0.0244$$
$$R^2 = 0.2522$$

Figure 3. Paradermal section of the water hyacinth leaf showing the stomata.
(a) Upper surface, and (b) Lower surface. (Bar = 50 μm).

Figure 3 shows stomata on the upper and lower surface of water hyacinth leaf. Based on table 1, it shows that on the 4th test, the smaller the leaf surface area, the less the number of stomata, which then influences the lower rate of transpiration. The same event was also shown in the 2nd test. If seen based on the number of stomata, the possibility of the rate of transpiration is low because the number of stomata on the upper surface of the leaf is less than on the lower surface of the leaf. This is less consistent with the literature which says the number of water hyacinth stomata on the upper surface of the leaf is more than the subsurface of the leaf [5]. This study has also been proven (figures 1 and 2 that show a positive correlation. Based on the 3rd and 5th tests, it showed a high stomata index due to the number of stomata is more than epidermal cells so that it can accelerate the evaporation of water from plants. Table 1 shows that the number of stomata at 1st, 3rd and 5th replications are in accordance with the literature which shows that the number of stomata is more on the leaf surface. This is one form of adaptation of aquatic plants to accelerate the evaporation process so that physiological temperatures in plants are better maintained [4]. In addition to leaf surface area, length roots affect the transpiration which is then related to the amount of water absorption by the root [14]. Trichomes on the leaves can prevent an excessive evaporation. In addition, evaporation can be reduced by the formation of a cuticle layer on the leaf surface which is quite thick. However, the water hyacinth plant does not have trichomes on the leaf surface. The waxy layer develops preventing the loss of water because of an intense heat [15].
Besides the number, the size of the stomata also determines the rate of transpiration. Stomata with a large size have a relatively higher transpiration rate [16]. Based on the research [4], the water hyacinth stomata has a length of 0.0475 mm and a width of 0.035 mm with the type of paracytic. The size of the water hyacinth stomata is the largest compared to other aquatic plants such as water spinach, lotus, yellow velvetleaf, and water jasmine. Stomatal opening is influenced by carbon dioxide, light, humidity, temperature, wind, leaf water potential and the rate of photosynthesis. In most plants, light and moisture in the low leaves make the guards cells lose their turgor, resulting in closure of the stomata [17]. Stomata are very helpful in terms of CO₂ absorption for photosynthesis. The distribution of stomata is closely related to the speed and intensity of transpiration in the leaves. For example, the location of one another with a certain distance within a certain limit, the more portions the faster the evaporation. If the holes are too close together, then evaporation from one hole will inhibit the evaporation of the nearby holes. This is because the path taken by the water molecules passing through the hole is not straight but turn aside due to the influence of the corners of the closing cells [13].

3.2. External factors that affect transpiration rates

The process of opening and closing the stomata is greatly influenced by light. Sunlight can affect the rate of transpiration in two ways, first, the light will affect the temperature of the leaf, so that it can affect transpiration activity and second, it can affect transpiration through its effect on opening and closing the stomata [14]. Rays contain heat, especially infra-red rays which can raise the temperature. The higher the intensity of sunlight, the higher the air temperature, so that the stomata will open wide which makes the transpiration rate higher.

In sunny conditions, the air does not contain much water. In these conditions the vapor pressure inside the leaf is much higher than the vapor pressure outside the leaf, so that water molecules diffuse from a high concentration (inside the leaf) to a low concentration (outside the leaf) so as to launch a transpiration [18]. Therefore, humidity affects the transpiration rate greatly. The lower RH air will further accelerate the rate of transpiration because water vapor will move from having high pressure (leaves) to low pressure (air) [16]. If the leaves have sufficient water content and open stomata, the rate of transpiration depends on the difference between the concentrations of water vapor molecules in the intercellular cavity in the leaves with the concentration of water vapor molecules in the air [19].

The wind has a dual influence that tends to conflict with the rate of transpiration. The wind carries the transpiration water vapor, so the wind decreases the humidity of the air above the stomata. However, if the wind sweeps the leaf surface, it will affect the temperature of the leaf. The temperature of the leaves will decrease and this can reduce the rate of transpiration. The rate of transpiration can be influenced by the availability of groundwater and the rate of absorption of water in the roots. During the day, water is usually transmitted faster than water absorption from the ground. This causes a deficit of water in the leaves, resulting in a large absorption at night. If the availability of groundwater decreases due to absorption by the roots, then the movement of water through the soil into the roots becomes slow. This tends to increase the water deficit in the leaves and decrease the rate of transpiration. Deeper rooting increases water availability and root proliferation increases the uptake of water from a unit of soil volume before plant withering [19]. In addition, the pH of water also affects the opening and closing of stomata. An increase in environmental pH stimulates phosphorylase to convert starch into glucose. An increase in osmose so that water enters from neighboring cells to cover cells, increasing volume causes turgor, so porous stomata are opened [13].

4. Conclusion

Based on the results of the study, the effect of leaf surface character on the ability of water hyacinth to transpire water can be summarized as: (1) the lowest leaf surface area is correlated with number of stomatal and transpiration rates in the individual measured plants. (2) External factors: the intensity of sunlight, temperature, humidity and wind speed affect the internal factors such as the number of stomata,
the size of the stomata and the process of opening and closing the stomata which can affect the speed of the transpiration rate.

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