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Wetting Properties and Foliar Water Uptake of *Tillandsia* L.☆

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A R T I C L E I N F O

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A B S T R A C T

Quantitative dimensional analyses of the wetting property of selected *Tillandsia* L. were conducted. The wettability on the leaf surfaces of three *Tillandsia* species and one hybrid cultivar has significant variations ($p < .05$). This variation is influenced by their absorptive foliar trichomes. The structure, arrangement and density of their foliar trichomes on the leaf surfaces and the degree of corrugated trichome wings with variations on micro−/nano-protrusion allow the liquids to increase its spreading and/or liquid repellency. Among the *Tillandsia* species, *T. schiedeana* Steudel has the densest trichomes. The average trichome densities are as follows: *T. schiedeana* (61.20 mm^2 ± 3.36) has the highest and *T.* Houston (*T. stricta* Sol. ex Sims *T. recurvifolia* Hook) hybrid (45.24 mm^2 ± 5.93) has the lowest trichome density on the adaxial leaf surface; while *T. schiedeana* (63.55 mm^2 ± 10.46) has the highest and *T. xerographica* Rohweder (40.66 mm^2 ± 17.72) has the lowest trichome density found on the abaxial leaf surface ($p < .0001$). All examined *Tillandsia* exhibited foliar water uptake. One of them, *T. schiedeana* had significantly greater increase in leaf water content up to 115.9% followed by *T.* Houston (57.37%) > *T. xerographica* (36.63%) > *T. caput-medusae* E. Morren (35.91%). Based on the results of adhesion and surface free energy of the leaf surfaces, the desirable wetting properties of all four *Tillandsia* plants used in this study were determined. Among the four, *T. schiedeana* and *T. caput medusae* exhibited interesting liquid adhesion on the adaxial leaf surface which makes the two plants hydrophilic on this particular leaf surface. On the other hand, the highest water drop adherence to the leaf surface is observed in *T. schiedeana* which is necessary for its high foliar water uptake. In this study, it was proven that structure, arrangement and density of foliar trichomes found in *Tillandsia* affect the spreading of liquid and leaf surface wettability on their leaf surfaces which in turn improve the foliar water uptake of these plants.

1. Background

Water is a significant resource that everyone of us need to survive yet roughly over a billion people in the world still does not have access to reliable sources of this very important resource [1]. In some dry regions, however, fogs are common which can percolate into moisture that arid plants absorbed through their leaves [2]. Recently, fog collection has become an alternative source of water for agricultural, industrial and domestic uses in these regions [3] and several emerging technologies are enabling human populations living in these regions to capture fog from the atmosphere [4]. Although there are already well-founded experimental designs and models available for fog collection, their methods and performance can still be improved. Knowledge gained from bio-inspired research, for instance, may serve as a guideline for their rational redesigning with new features and engineering concepts. Such is the underlying motivation, why biological surfaces with their unique microstructures and wetting properties are used as bases for biologically inspired moisture-absorbing surfaces [5,6].

Wettability of surfaces have received tremendous interests both from fundamental and applied points of view [7]. This property plays a significant role in many industrial processes, such as oil recovery, lubrication, printing, and spray quenching [8–11]. Wettability studies usually involve the measurement of contact angles as the primary data, which indicates the degree of wetting when a solid material surface and liquid component interacts with each other [12]. In such cases, small contact angles ($< 90^\circ$) correspond to high wettability, while large

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contact angles (> 90°) correspond to low wettability. For water, surface with high wettability may also be termed as hydrophilic while a surface with low wettability as hydrophobic [13].

In recent years, there has been a growing interest in the study on both superhydrophobic and superhydrophilic biological surfaces, mainly due to their potential applications in electrowetting, nanofluidics and self-cleaning [14–18]. However, wettability on biological surfaces was only first observed on the water repellent leaves of sacred lotus (*Nelum bonucifera* Gaertn.) which became the keystone in the self-cleaning mechanism of many biological surfaces [19]. Since then, micro − /nano-structures of many of these biological surfaces were the inspirations, bringing new insights to hydrophobic and hydrophilic industrial applications [5,6,20]. Notably, one of these motivations for hydrophobic and hydrophilic biological surfaces is the tuning of a solid surface to control water droplet's movement, which can find many applications in the fields of engineering, biology, and microfluidics.

Water interception to biological surfaces is due to the bond between the liquid and the solid. This phenomenon is caused primarily with the degree of surface structure in the micro/nano-level [19]. Hence, most air plants have unique surface structures which allow water to adhere. One interesting plant group that has this unique property is the plant genus called *Tillandsia* L. This genus of mainly epiphytic and arid plants has unique foliar trichomes [22,23] capable of absorbing water and nutrients from atmospheric moisture [24,25]. The role of these trichomes in *Tillandsia* species is very important for water retention especially during the period when moisture content in the atmosphere is low. During this low-moisture condition, the trichome wings in *Tillandsia* leaves are elevated. However, when a condition turns moist, the wings fold and stick to the leaf surface. This flattening of the trichomes on the leaves of *Tillandsia* caused a marked reduction in the contact angles resulting to liquid spreading over the surface of *Tillandsia* leaves. This phenomenon decreases liquid runoffs from the leaf surface [26–28].

Due to its unique trichome system, *Tillandsia* species has a remarkable foliar water uptake mechanism [21,29]. Most *Tillandsia* species plants are known for their preferences to arid conditions where air relative humidity and water saturation deficit are higher resulting in larger rates of moisture uptake [30]. However, the capacity for foliar water uptake among *Tillandsia* species is not well established. As an addition to their pivotal role in the absorption of atmospheric moisture, foliar trichomes in *Tillandsia* species also affect leaf wettability [29]. This situation is also observed in the leaf epidermis of other bromeliads having very dense trichomes [6].

The focus of this paper is water adherence to biological surfaces of epiphytic plants like *Tillandsia* species. The plants are good candidates for fog collection because they absorb most of their nutrients from the atmospheric moisture [21]. Although most *Tillandsia* species are known to be hydrophilic both on the adaxial and abaxial sides of their leaves [31], this species and its bromeliad relatives have rarely received attention for research and innovation. Hydrophilicity is unique to a few groups of plants like *Tillandsia* species which allows them to survive in arid habitats where fog is an important water source. Hydrophilic property of *Tillandsia* species needs to be further investigated to understand the physiological variations existing among its members especially in terms of water and nutrient absorption. Hence, in this paper, we analyzed the surface wettability and foliar water uptake of *Tillandsia* species. We also characterized the micro/nano-structures of their unique foliar trichomes. In addition, we also analyzed the surface wettability and foliar water uptake capacities of these foliar trichomes.

2. Methods

2.1. Plant Sampling

Three species and a hybrid cultivar of *Tillandsia* species: *T. xerographica* Rohweder; *T. caput medusae* E. Morren; *T. schiedeana* Steudel; and *T.* Houston Cotton Candy (*T. stricta* Sol. ex Sims x *T. recurvifolia* Hook), were commercially acquired from Insular Botanical International Inc., a local nursery located in Lucban, Quezon, Philippines. Each plant was divided into two sets each consisting of five replicates. One set (*Tillandsia* Set 1) was used for scanning electron microscope (SEM) examinations while the other set (*Tillandsia* Set 2) for foliar water uptake experiment and contact-angle (θ) measurements.

2.2. Acclimatization

Both *Tillandsia* Set 1 and *Tillandsia* Set 2 plants were acclimatized for 10 days in a small greenhouse under continuously controlled environmental conditions: temperature of 28-32 °C and relative humidity of 70–90%. During the acclimation period, the plants were laid over a 2-mm mesh-net platform constructed about 1 m above the ground. The plants were shaded under the foliage of trees to prevent photoinhibition. To standardize water availability across all *Tillandsia* plants, each was carefully sprayed with 50 ml of deionized water in the early evening for every 2 days including the day before the experiment commenced. Water spraying was done at least 2 h after sunset to simulate nocturnal fog exposure [31]. In other days, the plants were sprayed with 50 ml of distilled water. During their short acclimation period, the plants were carefully tendered and monitored to remove contaminants such as dust and wing scales of trapped insects without causing damage to the surface architecture of the plants. Prepared leaves from these plants were used for foliar water uptake and surface microstructure examination.

2.3. SEM Examination and Image Analysis of Microstructures

Three epidermal strips across distinct locations (tip, middle, and base) over the adaxial and abaxial surfaces of each leaf samples taken from each replicate of *Tillandsia* Set 1 were selected for structural examination of foliar trichomes. The epidermal strips were lyophilized and covered with gold using a sputter coater. The strips were examined under SEM (JEOL JSM-5310) at ×75, ×100 and ×200 magnifications with micrographs taken to get the leaf surface roughness of each sample.

Representative areas over the ×75 SEM micrographs of each epidermal strip were chosen based on systematic uniform random sampling [32]. These representative areas per strip were obtained to take samples consisting of five random measurements. Each representative area was fitted into a frame measuring 1 mm × 1 mm in area. The images were uploaded in ImageJ [33] to quantify dimensional analyses of microstructures [34] among the four *Tillandsia* plants.

The numbers of trichomes found within each frame was counted and mean of the total count for all frames per strip was computed. The computed mean is then used to calculate the trichome densities for each *Tillandsia* plant using the following formula:

$$Trichome\ density = \frac{No.\ of\ trichomes}{Leaf\ Area\ (mm^2)} \quad (1)$$

2.4. Foliar Water Uptake

Foliar water uptake capacity was evaluated according to the method used from the previous experiment with some modifications [35,36]. The whole procedure was conducted on all five replicates of *Tillandsia* Set 2 plants immediately after 2 h of post-sunset darkness simulating the typical conditions for nocturnal exposure. The starting mass ($Mass_1$,g) of the leaves for each replicate was measured using an electronic balance (0.001 g resolution) under the circumstance of wind-resistant shelter. The leaves were immediately submerged in deionized water with the basal ends above the water line to prevent water entry to the roots. These leaves remained submerged in darkness for 12 h to test potential foliar water uptake. The rehydrated leaves were drawn out of the deionized water and thoroughly patted dry with paper towels. Thereafter, the second mass was taken and recorded ($Mass_2$,g). To avoid residual water persisting on the leaf surface, the leaves were air-dried for 30 s and the third mass was measured ($Mass_3$,g). The leaves of *Tillandsia* are usually long and slender, rendering the calculations of the leaf area difficult. Therefore, the leaves were first removed from the plant stem and their fourth mass will be taken ($Mass_4$,g). Meanwhile, the mass of defoliated stem was measured to become the fifth mass ($Mass_5$,g). The detached leaves were dried at 105 °C for 0.5 h and then dried the second time at 80 °C for 24 h. The dry mass of these leaves was measured and recorded as the sixth mass (W_{Dry}, g).

The foliar uptake was standardized per dry mass (g). The initial weight (W_I,g) and the final weight (W_F,g) of leaves were calculated by (W_I) and (W_F) as follows:

$$W_I = Mass_1 - Mass_5 \tag{2}$$

$$W_F = (Mass_2 - Mass_4 + Mass_3) - Mass_5 \tag{3}$$

The initial leaf water content (LWC_I,%) and the final (LWC_F,%) was calculated, respectively:

$$LWC_I = \frac{W\mathrm{I} - W\mathrm{I}\,W\mathrm{Dry}}{W\mathrm{I}} \times 100 \tag{4}$$

$$LWC_F = \frac{W\mathrm{F} - W\mathrm{F}\,W\mathrm{Dry}}{W\mathrm{F}} \times 100 \tag{5}$$

Foliar water uptake was calculated with the amount by evaluating the change in leaf water content before and after submergence as:

$$Uptake = \frac{(Mass2 - Mass1) - (Mass4 - Mass5)}{(Mass1)} \times 100 \tag{6}$$

The increased percentage of leaf water content (*Rw*, %) with the given expression from previous research [36] were calculated using the following formula:

$$R_w = \frac{W\mathrm{F} - W\mathrm{I}}{W1 - W\mathrm{Dry}} \times 100 \tag{7}$$

Differences among *Tillandsia* Set 2 plants were analyzed using ANOVA to determine the significant increase of their leaf water contents.

2.5. Contact-Angle Determination

Contact-angle (θ) measurement for the five replicates of the four *Tillandsia* plants was conducted. This was done using ThetaLite 100 Tensiometer at the iNano Laboratory of Science and Technology Research Complex of De La Salle University. Measurement of contact-angle was performed on three separate locations (tip, middle, and base) over the adaxial and abaxial surfaces of each four leaf samples per replicate. A drop of five microliters (μl) each of the three liquid tests was used for contact-angle measurements, such as water and diiodomethane with different surface tension components. After 10 s, contact-angle (θ) measurements in each leaf surface were noted to determine if it has hydrophilic or hydrophobic interaction. There were 20 replicates in each location, and in both the adaxial and abaxial leaf surface. The mean values for the contact-angle measurements in three-liquid tests on the adaxial and abaxial leaf surfaces were analyzed statistically using One-way ANOVA.

2.6. Work-of-Adhesion and Free Surface Energy

Work-of-adhesion (W_a) and Surface free energy (SFE) were calculated using two liquids: deionized water and diiodomethane (99%, contains copper as a stabilizer; Sigma Aldrich-158,429). To measure the strength of interaction between solid surfaces (represented here by the *Tillandsia*'s abaxial and adaxial leaf surfaces) to the two liquids, drop adherence and liquid-solid interactions of each liquid were determined using the following Eq. [37]:

$$Wa = (1 + cos\theta)\gamma_l \tag{8}$$

Surface free energy (*SFE*), represented here by the symbol γ_s, was calculated using the Fowkes method [40,41]. These calculations were obtained from contact-angle measurements (θ) and the surface tension component of deionized water and diiodomethane [42] following the formula below:

$$\gamma_s = \gamma_s{}^{d} + \gamma_s{}^{p} \tag{9}$$

$$\gamma_s{}^{d} = 0.25\gamma_l(1 + \cos\theta) \tag{10}$$

$$\gamma_s{}^{p} = \{0.5\gamma_l(1 + \cos\theta^{p}) - (\gamma_s{}^{d}\gamma_l{}^{d})\,0.5\}2/\gamma_l{}^{p} \tag{11}$$

where γ_s – the experimentally determined leaf surface free energy, $\gamma_s{}^{d}$ – the dispersed component of leaf surface energy, $\gamma_s{}^{p}$ – the polar component of leaf surface free energy, γ_l- surface tension of the liquid against the leaf surface, θ – the contact angle between the dispersed component of leaf surface and the liquid, θ^{p} – the contact angle between the polar component of leaf surface and the liquid, $\gamma_l{}^{d}$ - surface tension of the liquid against the dispersed component of leaf surface, $\gamma_l{}^{p}$ - surface tension of the liquid against the polar component of leaf surface. Similarities among *Tillandsia* plants based on obtained trichome density, foliar water uptake and wettability values (Work-of-adhesion and SFE) were determined using the hierarchical clustering analysis.

3. Results and Discussion

3.1. Diversity of Foliar Trichomes

Under SEM examination, the individual foliar trichomes found on both adaxial and abaxial leaf surfaces of *Tillandsia* plants were similar in general architecture. They appeared as stalked asymmetric structures which are often wing-peltate in shape that end in shields with movable wings as what previously described [38]. The most significant part of the trichome is the shield as it plays an important role in capturing and collecting atmospheric moisture [21,29]. However, foliar trichomes of these *Tillandsia* plants do differ in terms of the positional arrangement, in minor structural details and trichome density (Fig. 1). The variations observed among the foliar trichomes of *Tillandsia* plants are summarized in Table 1.

The wings located in the shields of foliar trichomes were found distinct between examined *Tillandsia* plants. Notably, detailed examination of these wings from the foliar trichomes of each plant yielded very interesting findings (Fig. 1). On the adaxial surface, for instance, the trichome wings of *T.* Houston cotton candy (Fig. 1A) are elevated and the central disks are apparent. Both *T. xerographica* and *T. caput-medusae* (Fig. 1C, and E) have shorter trichome wings but the trichome wings of *T. caput-medusae* are more elevated in upright position compared to the trichome wings of *T. xerographica* which are less elevated and are partly tolled off. *T. schiedeana* (Fig. 1G) has the longest and thinnest trichome wings but similar to *T. caput-medusae* the trichome wings of *T. schiedeana* are also elevated in upright positions. On the abaxial side of the leaves, both *T.* Houston cotton candy and *T. schiedeana* (Fig. 1B and H) have the longest trichome wings. However, the trichome wings of *T.* Houston cotton candy are more elevated and overlaps each other compared to less elevated (oftentimes bent) trichome wings of *T. schiedeana* which never overlaps each other. Trichome wings of *T. xerographica* (Fig. 1 D) are the shortest but similar to *T.* Houston cotton candy its trichome wings are never overlapping. *T. caput-medusae* (Fig. 1 F) possessed the largest trichome wings over the abaxial side of the leaves among the four plants examined.

The edges of trichome wings also differ among the four *Tillandsia* plants examined. In Fig. 2A, *T.* Houston cotton-candy possesses sharply-pointed edges while that of *T. xerographica* in Fig. 2B has smooth pointed edges. The wing edges of *T. caput-medusae* (Fig. 2C) has soft

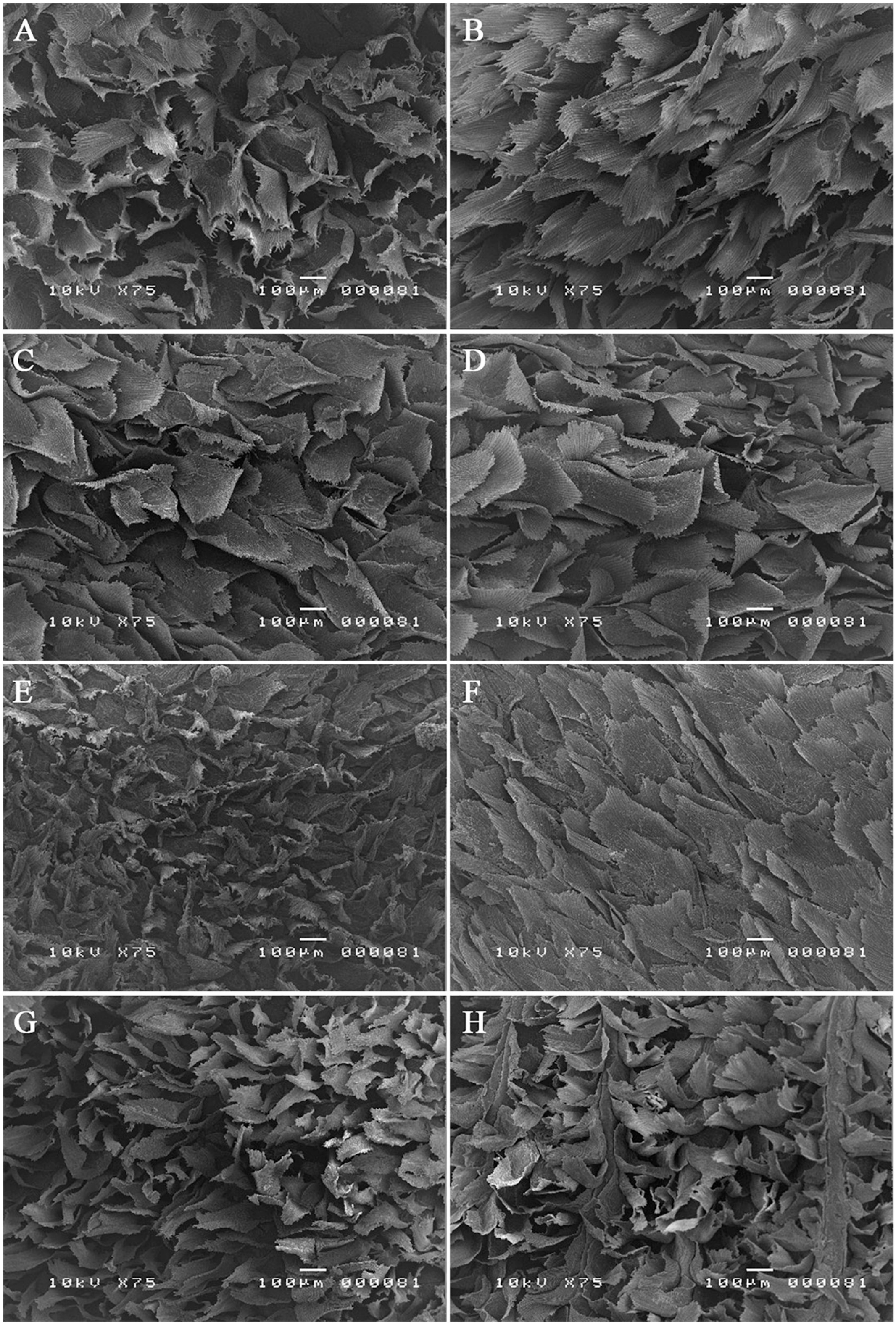

Fig. 1. Scanning electron micrographs of four examined *Tillandsia* species with 10 kV in ×75 magnification: *T*. Houston cotton-candy, (A) Adaxial and (B) abaxial leaf surfaces; *T. xerographica*, (C)Adaxial and (D) abaxial leaf surfaces; *T. caput-medusae*, (E) Adaxial and (F) abaxial leaf surfaces; *T. schiedeana*, (G) Adaxial and (H) abaxial leaf surfaces.

rounded edges while that of *T. schiedeana* has cupcake wrapper-like edges (Fig. 2D).

There is no doubt that these differences in shield structural architectures of foliar trichomes observed in *Tillandsia* are important to the survival of these plants as these variations can vary the micro-relief of the leaf surfaces. The corrugations in the wings, for instance, are necessary to enhance absorption of atmospheric moisture. Under scanning electron microscopy (SEM), these corrugated structures are like grooves and ridges that channels the collected moisture towards the central disc (Fig. 2). Perhaps, these variations of shield structures in *Tillandsia* could

Table 1
Description of the foliar trichomes on the adaxial and abaxial leaf surfaces of four *Tillandsia* species.

Species	Description	
	Adaxial	Abaxial
T. Houston cotton-candy	Trichomes wings are elevated and the central disc are apparent.	Trichome wings are longer elevated and overlapping to each other; Central discs are often obvious.
T. xerographica	Trichome wings are short, less elevated and partly tolled off.	Trichomes are short, partly elevated and overlapping to each other.
T. caput-medusae	Shorter trichome wings and elevated in an upright position.	Larger trichome structure and wings. Central discs are more visible.
T. schiedeana	Longer and thinner trichome wings structure. Wings are elevated in an upright position.	Trichome wings are longer, elevated partly bended and rolled off. Epidermis is visible.

be an important parameter in measuring differences in terms of capacity to capture atmospheric moisture among *Tillandsia* plants that need to be addressed in future research.

3.2. Trichome Densities

Calculation of trichome density yields surprising differences among the four *Tillandsia* plants examined. Results of these calculations are summarized in Table 2. Ranking from the highest to lowest, the average trichome densities on the adaxial leaf surface are as follows: *T. schiedeana* (61.20 mm^2 + 3.36) > *T. caput-medusae* (56.38 mm^2 + 19.71) > *T. xerographica* (50.91 mm^2 + 8.35) > *T.* Houston cotton-candy (45.24 mm^2 + 5.93). On the abaxial leaf surface, the average trichome densities are ranked as: *T. schiedeana* (63.55 mm^2 +10.46) > *T.* Houston cotton-candy (43.35 mm^2 + 5.93) > *T. caput-medusae* (42.14 mm^2 +12.42) > *T. xerographica* (40.66 mm^2 +17.72). In brief, *T. schiedeana* has the densest trichomes on both leaf surfaces among the four *Tillandsia* plants examined. However, only the abaxial leaf surfaces show significant variations among the *Tillandsia* plants examined (p-value < .05).

Variation in trichome density between the adaxial and abaxial surfaces of same leaves is only significant in *T. caput-medusae*. This is due to its adaxial leaf surface being significantly denser compared to its

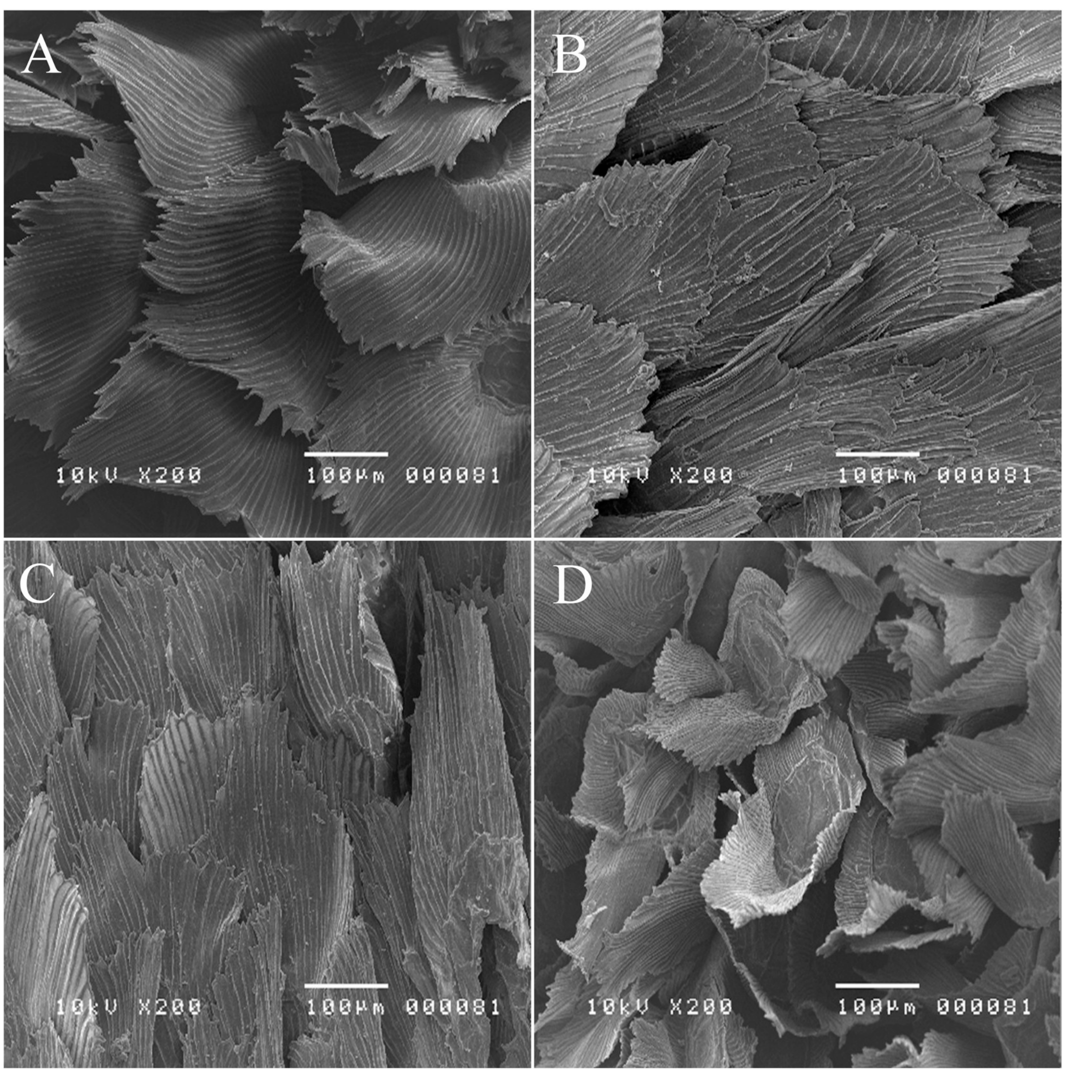

Fig. 2. Trichome wings of four *Tillandsia* species (with 10 kV in ×200 magnification) SEM examination: (A) *T.* Houston cotton-candy, (B) *T. xerographica*, (C) *T. caput- medusae*, (D) *T. schiedeana*.

Table 2
Trichome density on the adaxial and abaxial leaf surfaces of four *Tillandsia* species.

Species	Trichome density (No. of trichomes/mm^2)					
	Tip		Middle		Base	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
T. Houston cotton-candy	48.91 ± 3.78	51.52 ± 14.12	38.39 ± 7.85	43.01 ± 8.84	48.41 ± 6.45	35.53 ± 5.74
T. xerographica	56.48 ± 7.01	20.2 ± 2.31	54.93 ± 11.46	50.51 ± 11.96	41.31 ± 5.69	51.26 ± 8.39
T. schiedeana	57.87 ± 2.64	56.14 ± 7.64	64.59 ± 6.58	75.52 ± 6.31	61.15 ± 13.25	59.00 ± 5.27
T. caput-medusae	42.97 ± 9.47	28.04 ± 5.05	79.01 ± 5.33	51.45 ± 8.78	47.17 ± 15.52	46.94 ± 6.95

abaxial surface (p-value < .05). The plant body of *T. caput-medusae* is compact with strongly in-rolling (channeled) leaves which limit their adaxial leaf surfaces to light exposure. Furthermore, the channeled leaves of *T. caput-medusae* enable the trichomes on their adaxial surfaces to absorb more moisture during exposure to wet conditions. This is particularly, highest towards the tank-forming bases of the leaves where water can become stagnant during periods of prolonged wet conditions. The reduction of foliar trichomes on the abaxial leaf surface of *T. caput-medusae* is assumed to aid the plant in light reception. This leaf characteristic found in *T. caput-medusae* is also observed in shade-loving *T. bulbosa* and *T. butzii* with similar compact bodies and strongly in-rolled leaves [38,39].

The above findings also explain why atmospheric *Tillandsia* is able to adapt in arid regions. The dense foliar trichomes found on the adaxial leaf surface of *Tillandsia* are necessary for these plants to collect moisture from the atmosphere [26]. The abaxial leaf surface tends to have lower trichome density and the wings, though mobile, is less flexible compared to that of the adaxial leaf surface [38]. This characteristic reflects the adaptive radiation among species that exist in the arid region to allow their leaves, particularly the epidermis and stomates, to absorb more light for photosynthesis and gas exchange [36].

3.3. Foliar Water Uptake

All four *Tillandsia* plants demonstrated the capacity for foliar water uptake during 12 h submergence in deionized water. The foliar uptakes of these plants are as follows: *T.* Houston cotton-candy, 26.23 g > *T. xerographica*, 15.74 g > *T. caput-medusae*, 15.34 g > *T. schiedeana*, 11.18 g. However, leaf water content after submergence differs (Fig. 3) indicating that each plant has its own way of retaining water. In Fig. 4, water content increases significantly after submergence for the four *Tillandsia* plants with *T. schiedeana* showing the highest increase of water content (115.9%), followed by *T.* Houston cotton-candy (57.37%), *T. xerographica* (36.63%) and finally by *T. caput-medusae* (35.91%).

The plant bodies of *T.* Houston cotton-candy, *T. xerographica*, and *T. caput-medusae* are almost similar in the sizes and all have slender and

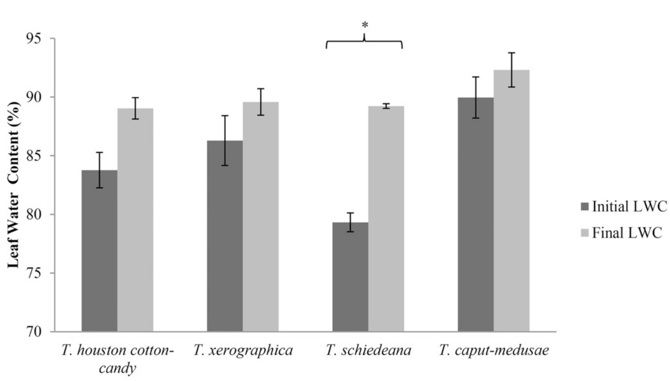

Fig. 3. The mean (± SE) leaf water content before and after submergence.

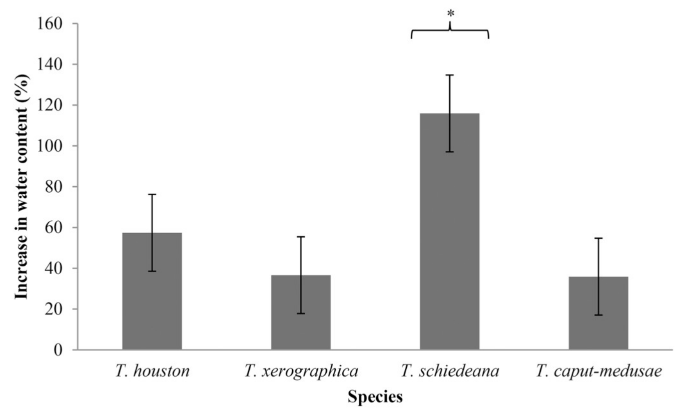

Fig. 4. The mean (± SE) increase in leaf water content.

thick leaves compared to *T. schiedeana* which has small and thin leaves (Fig. 2). Among the four plants, *T. xerographica* and *T. caput-medusae* exhibited a similar amount of water uptake with a difference of 0.4 g because both plants have similar masses before submergence.

In Fig. 4, *T. schiedeana* exhibited the highest increase in leaf water content despite its smaller body and thinner leaves. It also has the most significant increase in leaf water content in terms of percentage (*p*-value < .0001). This indicates that *T. schiedeana* has a very effective foliar water uptake compared to the other plants used in this study. The difference of foliar capacity between species is mainly attributed by the density of their trichome, the latter is highly effective at water and nutrient uptake [35]. Since *T. schiedeana* has significantly denser trichomes (See Table 2) it only indicates that despite its smaller size and shapes of leaves, foliar trichomes influenced the foliar water uptake of *T. schiedeana* which in turn increases the leaf water content of the plant to 115.9%.

Trichomes can be essential structures for leaf permeability to water. Leaf permeability to water has ecological significance through the interception of precipitation since water potential gradient moves water from the leaves into the internal tissues [36]. To the *Tillandsia*, the density of trichomes limits water loss [23]. This was specifically observed in *T. schiedeana*, because it has the highest increase in leaf water content. This could probably have been influenced by its very dense trichomes, the highest among the four *Tillandsia* plants used in this study. Furthermore, an increase in water uptake is also necessary for nutrient absorption in the leaf trichomes of *Tillandsia*, since they get their basic water source from absorbing atmospheric moisture in their natural environment. We need to further study how these trichome densities correspond to leaf surface permeability exhibited by various *Tillandsia* plants in their natural environment.

3.4. Contact-Angle Measurement and Leaf Surface Wetting Properties

Drops of deionized water (Fig. 5a) and diiodomethane (Fig. 6a) on the adaxial leaf surfaces of *T. caput-medusae* and *T. schiedeana* have lower contact-angle measurements particularly on the middle part of

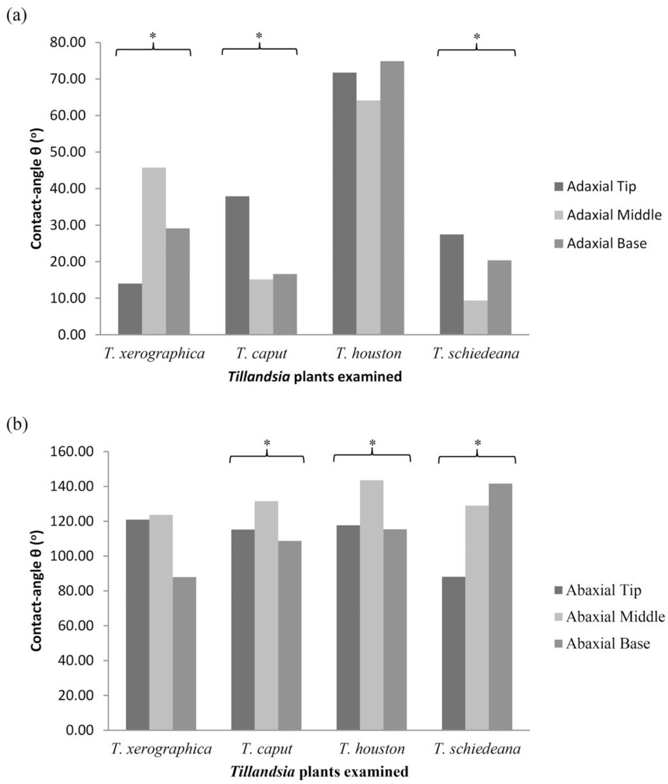

Fig. 5. Contact-angle measurement on the tip, middle and base of the adaxial (a) and abaxial (b) leaf surface of *T. xerographica, T.caput-medusae, T. Houston cotton candy* and *T. schiedeana* using deionized water. The (*) above the bars shows significant difference of CA in the tip, middle and base leaf parts having a *p*-value < .05.

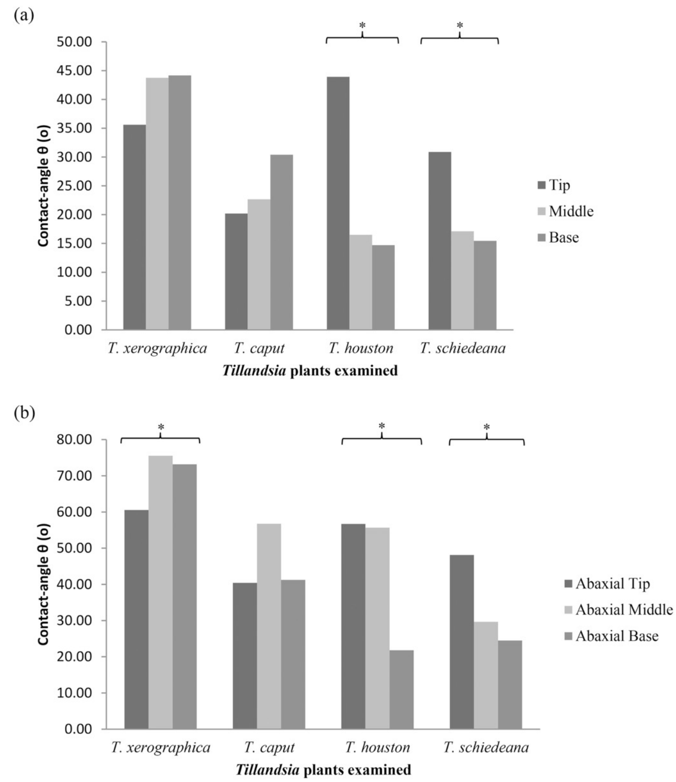

Fig. 6. Contact-angle measurement of the tip, middle and base of the adaxial (a) and abaxial (b) leaf surface of *T. xerographica, T.caput-medusae, T. Houston cotton candy* and *T. schiedeana* using diiodomethane. The (*) above the bars shows significant difference of CA in the tip, middle and base leaf parts having a *P* value < .05.

the leaves. This is probably due to the denser trichomes (Table 2) observed in these locations. The adaxial leaf surfaces of four *Tillandsia* plants, other than the middle portions of the leaves, all have less than 90^{o} contact-angle measurements hence, we considered these surfaces as having hydrophilic interactions (Fig. 7a, c, e & g) corresponding to high wettability and high liquid adherence [13].

In contrast, the abaxial leaf surfaces of these four *Tillandsia* plants have the higher contact-angle measurements in deionized water (Fig. 5) condition compared to adaxial leaf surfaces. Trichome wings on the abaxial leaf surface of *Tillandsia* plants tested are less flexible [26] which function to aid light reception on leaf epidermis and to form air pockets for allowing stomates perform gas exchange. The presence of air pockets between the trichomes and the rough leaf surface are thought to increase contact-angle measurements under deionized water condition. Surface with contact-angle measuring greater than 90^{o} is considered hydrophobic which leads to low wettability and low liquid adherence (Fig. 7b, d, f, & h) of the leaf surface [13].

Conversely, diiodomethane (Fig. 6a, b) drops on leaf surfaces of *Tillandsia* plants lead to higher contact-angle measurements. Diiodomethane is an apolar liquid and has higher dispersal component, hence, characterizing it as being nonpolar. Instead of adherence to the micro − /nano-structure of the corrugated leaf surfaces of the examined *Tillandsia* plants, diiodomethane tends to interact against the surface.

In summary, the abaxial leaf surfaces of four *Tillandsia* plants examined have higher contact-angle measurements for all liquid tests compared to their adaxial leaf surfaces. Using deionized water, the contact-angle measurement on the middle leaf parts of four *Tillandsia* species were higher including the base leaf part of *T. schideana*. On the diiodomethane liquid test, *T. xerographica* has higher contact-angle measurement particularly in the middle leaf part. Though it has a lower trichome number that would affect its wettability, (compared to *T. Houston cotton-candy* who has the lowest trichome density) its trichome wings structure influence the wettability. *T. xerographica* has shorter and more exposed trichome wings that would affect the cohesion of liquids (Fig. 1C and D) and has in-rolled trichome wing tips (Fig. 2B) where liquids would tend to interact against with the leaf surface.

The mean contact-angle (θ) measurements for deionized water and diiodomethane drops with different surface tension components on the adaxial and abaxial leaf surfaces of four *Tillandsia* species are summarized in Table 3. At the initial seconds, after the liquid drops, the CA (θ) values of water were high and tend to decrease after 10 s. This is due to the presence of the foliar trichomes of the examined *Tillandsia* species. However, in the case of diiodomethane the CA (θ) values were steady after 10 s of the liquid drop. These observations are due to the interaction of the two adjacent phases, the liquid and the leaf surface with varied structure and micro-relief.

Overall, using deionized water and diiodomethane, the adaxial leaf surfaces have lower contact-angle measurements compared to the abaxial leaf surfaces of the four examined species. In deionized water drops, all species exhibited hydrophilic adaxial leaf surfaces and hydrophobic abaxial leaf surfaces (p-value $\leq$.0001). On the other hand, using diiodomethane drops, contact-angle measurements were lower on the adaxial compared to the abaxial leaf surface and *T. schideana* shows to have the lowest CA in both leaf surfaces.

3.5. Work-of-Adhesion (W_a) and Surface Free Energy (SFE) Values

The work-of-adhesion (W_a) values or the values of work required for the adherence of deionized water and diiodomethane to the leaf surfaces are summarized in Table 4. In both liquids, *T. schiedeana* and *T. caput-medusae* showed higher work-of-adhesion (W_a) compared to *T.* Houston cotton-candy and *T. xerographica* on the adaxial leaf surfaces.

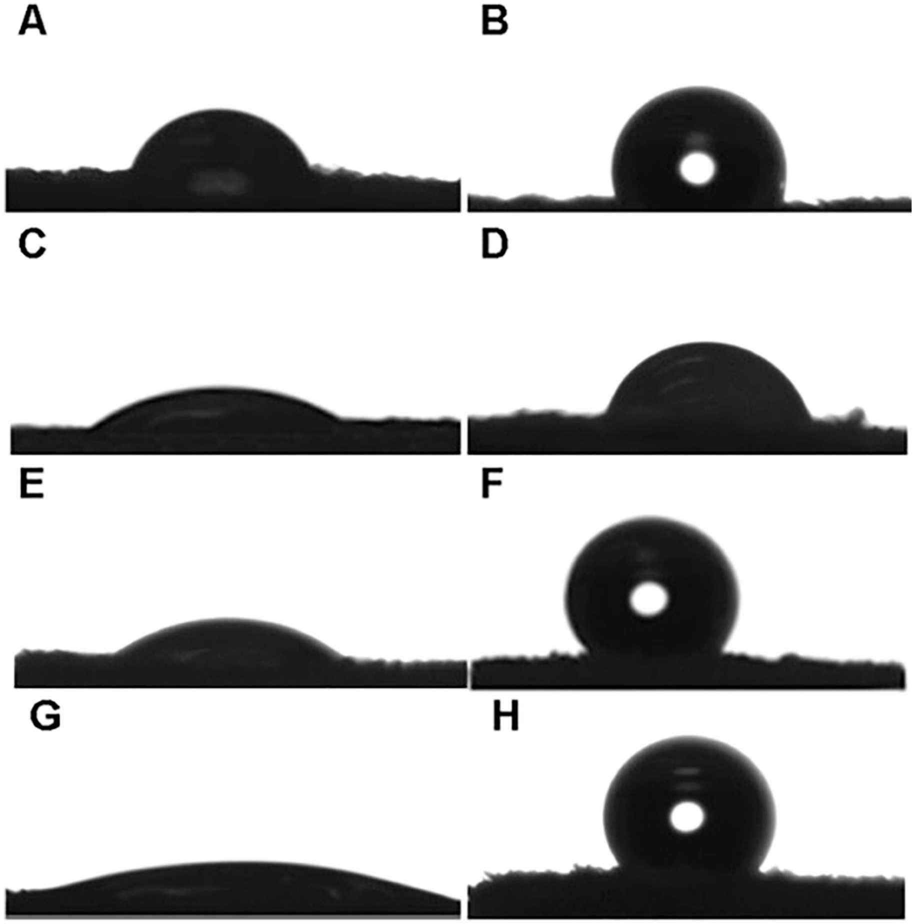

Fig. 7. Contact-angle of water drop on the leaf surface of four examined *Tillandsia* species with 10 kV in ×75 magnification: *T.* Houston cotton-candy, (A) adaxial and (B) abaxial; *T. xerographica*, (C) adaxial and (D) abaxial; *T. caput-medusae*, (E) adaxial and (F) abaxial; *T. schiedeana*, (G) adaxial and (H) abaxial.

Table 3
Mean values of contact-angle measurements using deionized water and diiodomethane on the adaxial and abaxial leaf surfaces of four *Tillandsia* species.

Species	CA θ			
	θ_w(°)		θ_d(°)	
	Adaxial	Abaxial	Adaxial	Abaxial
T. houston	70.24 ± 5.53	110.84 ± 19.88	25.04 ± 16.37	44.72 ± 19.86
T. xerographica	29.62 ± 10.88	120.03 ± 6.14	41.17 ± 4.83	69.75 ± 8.06
T. schiedeana	19.06 ± 9.12	120.35 ± 28.91	21.15 ± 8.47	34.09 ± 12.43
T. caput-medusae	23.21 ± 12.73	118.48 ± 11.76	24.41 ± 5.33	46.14 ± 9.19

Table 4
Work-of-adhesion (*W*a) of deionized water and diiodomethane of the adaxial and abaxial leaf surfaces of Tillandsia species.

Species	$W_{a,w}$ (mJm^{-2})		$W_{a,d}$ (mJm^{-2})	
	Adaxial	Abaxial	Adaxial	Abaxial
T. Houston cotton-candy	97.41	46.90	51.28	86.89
T. xerographica	136.47	36.37	58.45	68.38
T. schiedeana	141.61	36.02	100.18	92.87
T. caput-medusae	139.71	38.09	92.58	86.00

Table 5
Surface free energy of water and diiodomethane of the adaxial and abaxial leaf surfaces of Tillandsia species.

Species	γ_s (mJ/m^2)	
	Adaxial	Abaxial
T. Houston cotton-candy	52.80	37.65
T. xerographica	68.65	23.36
T. schiedeana	76.41	45.47
T. caput-medusae	74.76	37.93

On the abaxial leaf surfaces, *T. xerographica* has the lowest while *T. schiedeana* has the highest work-of-adhesion (W_a) compared to *T.* Houston cotton-candy and *T. xerographica*.

W_a measures the strength of interaction between the liquid and the leaf surface, and the energy released necessarily in the process of wetting. Hence, if the value of W_a is greater, the liquid spreads on the surface. This is essential for foliar water uptake to occur in the surface of leaves of *Tillandsia* as observed in this study.

Results of the calculations of surface free energy (SFE) are shown in Table 5. On the adaxial leaf surfaces, SFE values of both *T. schiedeana*

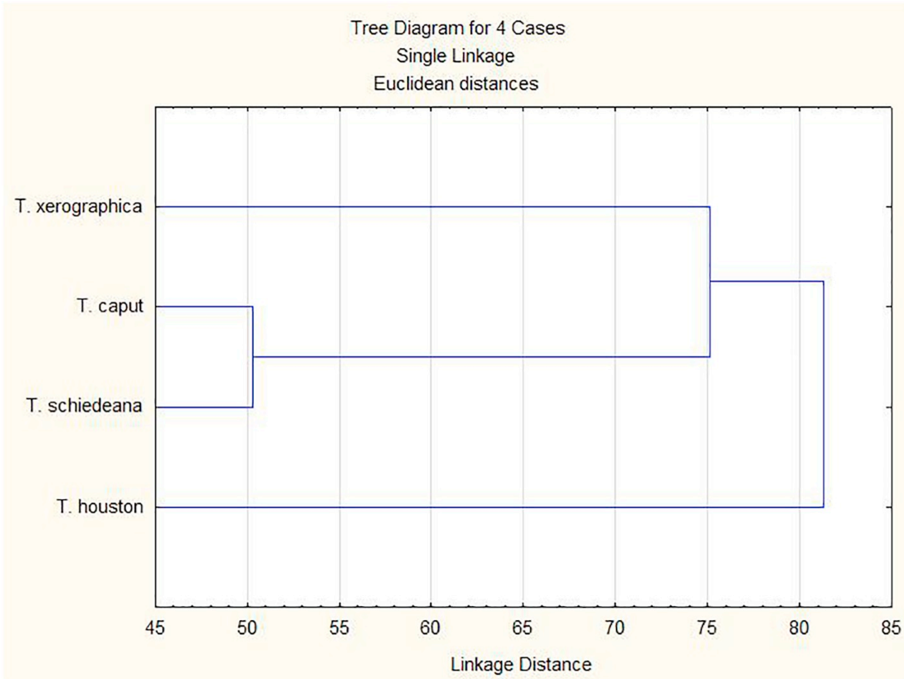

Fig. 8. Single-linkage Hierarchical Clustering Tree of the four *Tillandsia* species sharing the wettability properties and foliar water uptake together with the trichome density.

and *T. caput-medusae* are higher compared to *T.* Houston cotton-candy and *T. xerographica*. Similarly, on the abaxial leaf surfaces, the SFE values of *T. schiedeana* and *T. caput-medusae* are also higher compared to *T.* Houston cotton-candy and *T. xerographica*. SFE is a practical approach in estimating the energy released by the leaf surfaces. Its value is directly proportional to the calculated work-of-adhesion (W_a) of deionized water and diiodomethane. Thus, a higher SFE value means more wettable leaf surfaces for *T. schiedeana* and *T. caput-medusae*.

The calculated SFE on the abaxial leaf surfaces was inversely proportional to the calculated value of work-of-adhesion (W_a) of deionized water but directly proportional to diiodomethane. Diiodomethane has dispersion component only on its surface tension and it greatly influences the calculation for SFE [34]. Looking back on the contact-angle measurements of diiodomethane on the abaxial leaf surfaces, the values shows to be inversely proportional to the calculated work-of-adhesion (W_a) and computed SFE. Hence, the abaxial leaf surface of *T. xerographica* is more hydrophobic compared to the other species.

The linkage tree resulting from the hierarchical clustering analysis of four *Tillandsia* plants based on obtained values of trichome density, foliar water uptake, and wettability values are shown in Fig. 8. The tree reveals two branching. One of the branches clustered *T. xerographica*, *T. caput-medusae*, and *T. schiedeana* altogether. In this same branch, *T. caput-medusae*, and *T. schiedeana* are most similar in all values analyzed compared to *T. xerographica*. The other branch linked is *T.* Houston cotton-candy to the *T. xerographica*, *T. caput-medusae*, and *T. schiedeana* cluster. In our analysis, we considered *T. schiedeana* and *T. caput-medusae* as having the most desirable wetting property among the four *Tillandsia* plants we examined.

4. Conclusion

For the first time in study, the quantitative dimensions regarding the wettability of the water absorbing leaves of *Tillandsia* plants are provided. The wettability property exhibited by *Tillandsia* plants provided us baseline information on the diverse nature of their biological surfaces and these aids the plants to adapt and survive in arid regions. The wettability on the leaf surfaces of four *Tillandsia* plants has significant variations. This variation is influenced by their absorptive foliar trichomes. The structure, arrangement, and density of their foliar trichomes on the leaf surfaces and the degree of corrugated trichome wings with variations on micro−/nano-protrusions allow the liquids to increase its spreading and/or liquid repellency. The unique micro−/nano-structure of the trichome wings that influence interesting wetting and liquid adherence exhibited by the leaf surfaces of the four examined *Tillandsia* species will attract future researchers for innovations of bioinspired materials. Many of the interaction with the surrounding media depend on the value of the surface free energy, such as adsorption, wetting and adhesion. Its knowledge is therefore important for processes such as coating, painting, cleaning, printing, hydrophobic or hydrophilic coating, bonding, dispersion and many more.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biotri.2019.100103.

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