Decoding Leaf Wettability: The Role of Physicochemical Leaf Traits in Hydrophytic Plants

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Abstract

Leaf wettability refers to the extent to which water adheres on leaf surfaces., which can have positive or negative effect on leaf, plant and ecosystem functioning. Hydrophytic plants have developed morphological adaptations to cope up with the excess presence of water. In this study, we analyzed contact angles and key physicochemical leaf traits including length, width, area, thickness, water adhesion, stomatal density, trichome density, surface structure, and surface free energy of nine wetland plant species belonging to three different habitats i.e. floating, submerged and emergent, on the basis of leaf position, from the Western Ghats of Maharashtra. Measurements were taken for both the adaxial and abaxial surfaces to determine the correlations between these traits and leaf wettability. Among the examined traits, surface free energy emerged as the primary determinant of hydrophobicity, exhibiting a strong negative correlation with contact angle. Contact angles ranged from 61° to 143° on the adaxial surface and from 47° to 124° on the abaxial surface. While most other physical traits did not show significant correlation, the presence of trichomes in *Pistia stratiotes* a floating hydrophyte enabled the formation of water beads on its surface. Additionally, Scanning Electron Microscopy (SEM) was employed to investigate the microstructural and nano-structural features of both sides of the leaf, providing further insights into their role in wettability. Studying the adaptations of the hydrophytes to their environment reveals that incorporating wettability and nano structural surface traits, along with other plant characteristics, enhances our understanding of plant–water interactions in wetland ecosystems.

Introduction

Leaf wettability refers to the interaction of water with respect to the leaf surface, either by spreading or forming droplet, primarily measured by by the contact angle (CA) formed between a water droplet and the leaf surface. A high contact angle (greater than 90°) indicates hydrophobicity, leading to the formation of water beads that quickly shed from the surface, whereas a low contact angle (less than 90°) signifies hydrophilicity, allowing water to spread across the surface (Koch and Barthlott 2009). According to Aryal and Neuner (2010) and Wang et al. (2014) the wettability of leaves can be classified as follows: Leaves with contact angles less than 40° are categorized as super hydrophilic, reflecting an exceptionally strong affinity for water. Surfaces with contact angles between 40° and 90° are regarded as highly wettable. When the angle lies between 90° and 110°, the surface is considered wettable, whereas angles from 110° to 130° suggest moderate hydrophobicity. Leaf surfaces exhibiting contact angles in the range of 130° to 150° are identified as hydrophobic, while those exceeding 150° are termed superhydrophobic, indicating pronounced water repellency. This classification system provides a useful framework for comparing the wettability of different plant species under varying environmental conditions. This property is influenced by both the chemical composition and the micro- to nanostructure of the leaf surface, including the presence of epicuticular waxes and specialized structures like trichomes (Bhushan and Jung 2011).

With the rise in extreme weather events like heavy rainfall and droughts (Allan 2011), the interaction between leaves and atmospheric water has become increasingly relevant. Plant leaves not only interact directly with rainfall but also serve as condensation surfaces for fog and dew, thereby influencing the manner in which water reaches the ground (Dunkerley 2020). The retention of surface water on leaves not only alters the hydrological cycle but also has a diverse impact on the plant itself (Dawson and Goldsmith 2018). The effects on plants are both positive and negative. Depending on the conditions surface water retention can block transpiration and photosynthesis (Aparecido et al. 2016,2017; Berry and Goldsmith 2020) whereas in some cases foliar water uptake can boost photosynthesis and growth (Eller et al. 2013; Carmichael et al. 2020). The formation of water films on leaf surfaces can obstruct stomatal openings, thereby impeding CO_2 diffusion and reducing photosynthetic efficiency (Smith and McClean 1989). Given that CO_2 diffuses approximately 10,000 times slower in water than in air, even thin water layers can significantly limit gas exchange (Brewer 1991). Water films can alter the leaf's boundary layer, affecting transpiration rates. While a continuous water film may reduce transpiration by increasing resistance to water vapor diffusion, it can also lead to uncontrolled water loss if the cuticle is compromised (Brewer and Smith 1997). In epiphytic Bromeliads, wettable leaves play a specialized role in absorption of water and nutrient (Zambrano et al. 2019). Hydrophobic leaf surfaces can deter pathogen establishment by minimizing water retention, which is essential for the germination of many fungal spores. Conversely, hydrophilic surfaces that retain water may create favorable conditions for pathogen proliferation (Holder 2007). The "lotus effect," characterized by superhydrophobic surfaces with self-cleaning properties, allows water droplets to remove contaminants and pathogens as they roll off the leaf, thereby maintaining optimal photosynthetic surfaces and reducing disease incidence (Barthlott and Neinhuis 1997). Now in general, it appears that water presence for brief periods on the leaf surfaces can be beneficial but prolonged wetness often proves disadvantageous. Therefore, the balance of leaf wettability is crucial, with adaptations varying depending on environmental conditions and ecological niches.

Wetland habitats are characterized by unique environmental conditions such as constant or periodic waterlogging and high humidity. These conditions pose significant challenges to plants, including reduced gas exchange due to water films on leaf surfaces and increased susceptibility to pathogens that thrive in moist environments (Reynolds et al. 1989; Evans et al. 1992; Huang et al. 2006). To cope with excessive water, wetland plants have evolved various morphological and physicochemical adaptations. One crucial adaptation is the development of leaf surface traits that influence wettability, thereby affecting water retention and promoting efficient water shedding (Sikorska et al. 2017). The leaf wettability depends upon various factors like the epicuticular wax layer thickness and composition which play a major role in minimizing leaf wetting (Holloway 1969; Koch et al. 2006). Trichomes with higher densities often enhance hydrophobicity (Brewer et al. 1991; Pan et al. 2021). Stomatal density and spatial arrangement affect surface roughness and influence contact angle measurements (Brewer and Nuñez 2007; Wang et al. 2010). Surface free energy (SFE) affects the interaction between water and the leaf, with lower SFE correlating with higher hydrophobicity (Wang et al. 2014). Additionally, nano- and microstructural features like wax crystals and surface ridges further enhance water repellency by increasing roughness and modifying surface chemistry (Sikorska et al. 2017).

So, in the previously done research on the wettability of plant leaves from various habitats the focus has been given to individual trait or general principle of wettability rather than systematically correlating multiple physicochemical and structural characteristics with wettability across diverse wetland taxa. In this study, we investigate the relationship between leaf wettability and key physicochemical traits in nine wetland (aquatic) plant species native to the Western Ghats of Maharashtra, India. Both adaxial and abaxial surfaces were assessed for contact angles and also analyzed a set of traits including leaf length, width, length to width ratio, area, thickness, stomatal and trichome densities, water adhesion, surface structure, and surface free energy. Scanning electron

microscopy (SEM) was employed to examine micro- and nano-scale surface features. This study aims to identify the key leaf traits influencing hydrophobicity and to investigate their role in facilitating adaptive strategies in wetland plant species.

Materials and Methods

Using random sampling method for the selection, nine pond dwelling aquatic plants were selected for analyzing leaf wettability and the factors influencing it. The young and fully matured leaves after collecting were transported to the laboratory in the air tight packets so that they remain as fresh as possible. A total of 5 leaves were analyzed per plant species to determine the selected traits used in this study. Leaf wettability was determined measuring the contact angles of both adaxial and abaxial surfaces by the sessile drop method with the help of goniometer (rame hart goniometer). A 10 µl droplet of distilled water was deposited on the leaf surface employing a syringe and contact angle was determined by analyzing the image captured using CA meter software. For calculating surface free energy, we used 1-bromonapthalene as the nonpolar solvent. Using the Owens and Wendt (1969) method where the total surface free energy is divided into two components: a dispersive (non-polar) component and a polar component, surface free energy was estimated by measuring the contact angles of at least two different liquids typically one polar (distilled water) and one non-polar (1-bromonapthalene) whose surface free energy is known on the solid surface. The formula to calculate SFE is as following:

$$\gamma_s = \gamma_s^d + \gamma_s^p$$

$$\gamma_l(1 + cos\,\theta) = 2\left(\sqrt{\{\gamma_s^d \cdot \gamma_l^d\}} + \sqrt{\{\gamma_s^p \cdot \gamma_l^p\}}\right)$$

γ_s : Total surface free energy of the solid, γ_s^d: Dispersive component of the solid's surface free energy, γ_s^p : Polar component of the solid's surface free energy, γ_l : $\mathrm{Total\ surface\ free\ energy\ of\ the\ liquid}$, γ_l^d: Dispersive component of the liquid's surface free, energy, γ_l^p: Polar component of the liquid's surface free energy, θ : Contact angle of the liquid on the solid

For surface structure analysis, air-dried leaf samples were placed on mounting stubs and gold sputtering is done, then imaged using a JEOL JSM-IT200 scanning electron microscope to assess structural differences in relation to contact angle (Pathan et.al. 2008). Leaf length and width were measured using a ruler, and fresh weight was recorded using a standard weight box. The length-to-width ratio was subsequently calculated. Leaf area was derived from length and width measurements, adjusted by a shape correction factor, following the method described by Schrader et al. (2021). Leaf thickness was measured using a micrometer gauge. Leaf adhesion on both the adaxial and abaxial surfaces was determined using the formula $\mathbf{W = (W_1 - W_0)/S}$, where W_1 represents leaf weight after wetting, W_0 is the weight before wetting, and S denotes leaf's surface area (Wang et al. 2014). Trichome density (trichomes/mm²) on both adaxial and abaxial surfaces was manually evaluated using a dissecting light microscope with observations taken from ten sites per leaf (Viz and Pacada 2022). Clear nail polish was thinly applied to both upper and lower leaf surfaces and left to dry. The dried film was peeled off with adhesive tape, mounted on slides, and examined under a light microscope to count stomata per mm²(Zhu et al. 2018). The parameters were analyzed for their correlation with the adaxial and abaxial contact angles to determine their effect on wettability. Pearson correlation coefficient was computed for each parameter against the contact angles of both surfaces, with statistical significance at $p < 0.05$, with the help of Statistica 10 software (StatSoft Inc., 2011).

Results

A total of nine wetland plant species belonging to three habitats i.e. submerged, floating, and emergent based on leaf's position, were assessed for various morphological and surface traits in relation to leaf wettability, as quantified by contact angle (CA) measurements on both the leaf surfaces. On the adaxial surface, contact angles ranged between 61° and 143°, while on abaxial surface they varied from 47° to 124°, indicating a broad spectrum of wettability among the sampled species. Out of the nine species examined, only three i.e. *Colocasia esculenta*, *Nelumbo nucifera* from emergent habitat and *Pistia stratiotes* from floating habitat exhibited hydrophobic properties (CA > 90°), whereas the remaining six species displayed hydrophilic surfaces (CA < 90°). Additionally, in all but one species assessed, the adaxial surface showed contact angle more than the abaxial surface. All other physicochemical parameters were measured whose values are mentioned in the Table 1. Pearson's correlation analysis was employed to evaluate the strength, direction and significance of the associations between leaf traits (e.g., width, trichome density, surface free energy) and contact angle values.

Table 1 shows the data of all the parameters studied. This data was then used to find out Pearson's correlation coefficient values between the traits of leaf studied and the adaxial and abaxial contact angles measured at $p < 0.05$, using Statistica 10 software.

Table 1
Leaf trait measurements of wetland plants used to assess correlations with contact angle an

Plant name	Habitat	Length ± SD (cm)	Width ± SD (cm)	L/W ratio	Weight ± SD (g)	Thickness ± SD(mm)	Area (mm^2)	Adaxial CA ± SD (°)	Abaxial CA ± SD (°)	Water adhesion ± SD (adaxial)	Water adhesion ± SD (abaxial)	Adaxial CA ± SD (°) nonpolar
Colocasia esculenta	emergent	18.68 ± 0.67	12.52 ± 0.13	1.49	3.768 ± 0.19	0.272 ± 0.007	16371	121.25 ± 2.1	95.55 ± 1.52	0.61 ± 0.23 g/m^2	1.22 ± 0.45 g/m^2	76 ± 3.42
Nelumbo nucifera	emergent	15.4 ± 0.64	18.46 ± 0.88	0.83	6.84 ± 0.27	0.318 ± 0.007	22458	143.8 ± 2.2	124.2 ± 1.8	0.97 ± 0.09 g/m^2	1.33 ± 0.31 g/m^2	62.2 ± 3.06
Typha angustifolia	emergent	144.02 ± 2.98	2.8 ± 0.30	51.43	7.36 ± 0.17	1.296 ± 0.018	25808	79.5 ± 2.4	76.45 ± 1.9	6.58 ± 0.39 g/m^2	7.05 ± 0.44 g/m^2	30.7 ± 2.01
Ipomoea aquatica	floating	9.02 ± 0.68	5.62 ± 0.07	1.6	0.94 ± 0.05	0.31 ± 0.007	3549	75.15 ± 0.58	47.3 ± 0.8	14.09 ± 0.42 g/m^2	19.72 ± 0.73 g/m^2	16.9 ± 1.25
Nymphaea nouchali	floating	21.96 ± 1.59	18.6 ± 0.316	1.18	11.49 ± 0.54	0.42 ± 0.006	32268	63.1 ± 0.8	52 ± 1.5	13.94 ± 0.83 g/m^2	16.42 ± 0.19 g/m^2	44.05 ± 2.79
Pistia stratiotes	floating	11.12 ± 0.23	8.36 ± 0.13	1.33	7.86 ± 0.13	0.718 ± 0.017	6322	95.64 ± 0.58	113.4 ± 0.7	36.38 ± 0.72 g/m^2	104.4 ± 0.87 g/m^2	73.1 ± 3.28
Cryptocoryne retrospiralis	submerged	23.12 ± 0.28	1.48 ± 0.074	15.62	1.63 ± 0.028	0.292 ± 0.011	2258	62.85 ± 1.75	54.6 ± 1.77	10.62 ± 0.76 g/m^2	15.5 ± 0.38 g/m^2	45.6 ± 2.58
Eriocaulon cuspidatum	submerged	4.1 ± 0.109	0.92 ± 0.09	4.45	0.208 ± 0.007	1.14 ± 0.048	268	84.2 ± 1.7	58.5 ± 2.2	52.24 ± 0.33 g/m^2	55.97 ± 0.67 g/m^2	11.1 ± 1.04
Hygrophila auriculata	submerged	6.24 ± 0.35	1.24 ± 0.048	5.03	0.22 ± 0.015	0.31 ± 0.011	511	61.8 ± 1.3	60.05 ± 1.8	29.41 ± 0.51 g/m^2	35.29 ± 0.29 g/m^2	34.2 ± 1.76

The data in Table 1 had been used to find out the correlation between the various leaf parameters and the measure of wettability i.e. contact angle. The correlation coefficient (r) values show how strong is the relationship between the two parameters taken into consideration.

of every parameter against adaxial contact angles. of every parameter against abaxial contact angle.

Figures 1 and 2 show Pearson's correlation matrix heatmaps representing the linear relationships between key leaf parameters and contact angles on the adaxial (Fig. 1) and abaxial (Fig. 2) surfaces. The color gradients reflect the strength (r-values) and direction of the correlations, with values ranging from − 1 (strong negative) to + 1 (strong positive).

Using these Pearson's correlation coefficient values we found out the significance of all the parameters affecting the contact angles i.e. wettability of the leaves. The values for Pearson correlation coefficient and p- values which shows significance of the correlation are mentioned in the Table 2.

Table 2
Pearson Correlation Coefficients (r) and p-values Between Leaf Parameters and Contact Angles.

Parameter	r-value (Adaxial)	p-value (Adaxial)	r-value (Abaxial)	p-value (Abaxial)
SFE abaxial			**-0.975**	**0.000**
SFE adaxial	**-0.80**	**0.010**		
Width	0.561	0.116	0.480	0.191
Water adhesion adaxial	-0.35	0.365		
Stomatal density abaxial			-0.36	0.345
Area	0.277	0.471	0.264	0.493
L/W ratio	-0.235	0.543	-0.11	0.782
Weight	0.197	0.611	0.393	0.295
Water adhesion abaxial			0.160	0.681
Stomatal density adaxial	0.15	0.703		
Thickness	-0.111	0.776	-0.010	0.979
Length	-0.094	0.810	0.02	0.961
Trichome density abaxial			0.47	0.203
Trichome density adaxial	0.017	0.966		
Note: Significant results ($p < 0.05$) are displayed in bold.				

Among all measured parameters, **surface free energy (SFE)** showed the strongest correlations with contact angle. SFE on both adaxial and abaxial surfaces was **negatively correlated** with contact angle values, indicating that leaves with lower surface energy exhibited higher water repellency. For example, *Colocasia esculenta*, with low abaxial SFE (33 mJ/m²), showed high contact angles (adaxial: 121.25°, abaxial: 95.55°), suggesting strong hydrophobicity. Conversely, *Ipomoea aquatica*, which had the highest abaxial SFE (59.3) exhibited the lowest abaxial contact angle (47.3°), reflecting greater wettability. No consistent or significant correlations were found between contact angle and leaf length, width, thickness, area, or weight. In this study, trichome densities did not show significant associations with contact angle, likely due to their low or absent values across most species. But in case of *Pistia stratiotes*, a floating hydrophyte, the hydrophobic behavior is the result of presence of trichomes (density more than 25 mm^{-2}) on both of its surfaces. Regarding stomatal density, no significant association between stomatal density and contact angle on either surface was found as per the Pearson correlation analysis.

Scanning electron microscopy (SEM) was used to examine the microstructural features of both adaxial and abaxial leaf surfaces for all nine wetland plant species. The SEM images revealed distinct variations in surface texture, presence/absence of trichomes, stomatal exposure, and cuticular structures, which were analyzed in relation to contact angle measurements to infer their influence on leaf wettability.The two hydrophobic species, *Colocasia esculenta* and *Nelumbo nucifera*, showed specialized surface features that are consistent with high contact angle values. The surfaces of these leaves displayed a hierarchical surface architecture, including micro- and nanoscale roughness combined with dense epicuticular wax crystals. In *Nelumbo nucifera (*Fig. 3; *cand d)*, papillate epidermal cells and dense wax tubules were observed—hallmarks of the "lotus effect," which is known to promote superhydrophobicity by minimizing contact area between water and the surface (Barthlott and Neinhuis 1997; Koch et al. 2009). Similarly, the morphology of *Colocasia esculenta (*Fig. 3; *a and b)*, leaf exhibits a two-tier surface structure characterized by hexagonal microcavities resembling a honeycomb-like pattern (Kumar and Bhardwaj 2020) with uniform wax coverage and elevated surface ridges that contribute to its water-repellent properties. The surface structures of *Pistia stratiotes (*Fig. 3;*e and f)* show the waxy trichomes that help bead up the water drops.

The other six plants' contact angle in this study is below 90 degrees showing their affinity towards water. Their SEM analysis revealed surface structures consistent with those hydrophilic surfaces reported in previous studies.

The scanning electron micrographs (Fig. 4a–l) reveal consistent structural adaptations among the studied wetland species, supporting their hydrophilic surface behavior. Across all samples, the absence of micro- and nanoscale hierarchical features, including wax crystal deposition, correlates with low contact angles and increased wettability. In *Nymphaea nouchali* (Fig. 4a, b), both leaf surfaces show an undulating and irregular epidermis with no epicuticular projections. This simple surface structure promotes close contact with water, enhancing hydrophilicity. Similarly, *Cryptocoryne retrospiralis* (Fig. 4c, d) has an uneven surface with sunken stomata but lacks wax crystals, which likely aids in water absorption and gas exchange in moist or submerged environments. *Ipomoea aquatica* (Fig. 4e, f) exhibits a smooth surface with fine cuticular striations, which may help water spread evenly while reducing air trapping. The absence of wax further supports its wettable nature. In *Hygrophila auriculata* (Fig. 4g, h), the stomata are located in shallow depressions surrounded by smooth cuticular ridges, likely guiding water movement without blocking gas exchange. *Eriocaulon cuspidatum* (Fig. 4i, j) shows a mostly uniform surface with slight striations near the stomata, consistent with water-retaining surfaces adapted for diffusion-based gas exchange. Lastly, *Typha angustifolia* (Fig. 4k, l) displays uneven, micropatterned surfaces without waxes, suggesting a structural adaptation that maintains hydrophilicity while providing surface stability in wet habitats.

Discussion

This study investigated the relationship between leaf surface traits and wettability in nine wetland plant species by analyzing contact angles on both adaxial and abaxial surfaces. The results revealed a broad spectrum of wettability, with only three species exhibiting hydrophobic characteristics and the remainder showing hydrophilic behavior. Surface free energy (SFE) was found to be the most consistent predictor of contact angle, while traits like stomatal and trichome density showed inconsistent or weak correlations. These findings provide insight into how structural and physicochemical leaf traits contribute to ecological adaptations in wetland environments.

Hydrophobicity and leaf positioning

Interestingly, except *Pistia stratiotes*, the floating hydrophyte, whose hydrophobic behavior is attributed to the trichomes present on it, the other hydrophobic species are characterized by leaves that are held above the water surface rather than being submerged or floating. This leaf position suggests an ecological adaptation that favors water repellency, likely to facilitate self-cleaning (lotus effect) and prevent prolonged water retention, which can block gas exchange or promote microbial colonization (Barthlott and Neinhuis 1997; Koch et al. 2009). The elevated and hydrophobic leaves of *Nelumbo nucifera* are particularly well-known for their superhydrophobic nature, attributed to hierarchical surface structuring and wax crystals, which significantly reduce surface free energy and promote water rolling (Bhushan and Jung 2006). Similarly, *Colocasia esculenta* has been reported to exhibit a highly water-repellent surface, potentially serving to maintain gas exchange and light capture in wetland conditions (Ensikat et al. 2011). These surface adaptations can be viewed as a response to the plants' aerial leaf posture, which exposes them to rain and surface splash, increasing the need for self-cleaning mechanisms (Barthlott et al. 2007).

Adaptation of hydrophilic surfaces in aquatic plants

In contrast, the remaining hydrophilic species, often with submerged or floating leaves, do not exhibit such repellency. This observation supports earlier studies showing that there is reduced selective pressure for hydrophobic surfaces in aquatic or semi-aquatic environments where leaves remain consistently wet or underwater (Vogel 1988; Riedl and Riedl 2004). In such species, hydrophilic surfaces may even aid in water absorption or nutrient exchange, depending on environmental conditions. This surface polarity in wettability is well-documented in foliage, often reflecting functional specialization. The adaxial surface, being more exposed to environmental factors like rainfall and sunlight, may evolve more hydrophobic traits to facilitate rapid droplet shedding and self-cleaning. The abaxial surface, typically more shaded and involved in gas exchange through stomata, may maintain a more hydrophilic profile to support physiological functions (Neinhuis and Barthlott 1997; Brewer 1991).

Contribution of surface free energy to wettability

The inverse relationship between SFE and contact angle aligns with established principles of surface chemistry, where lower SFE facilitates water repellency (Bhushan and Jung 2011). This relationship emphasizes the significance of surface physicochemical properties in determining leaf wettability across species with different ecological strategies.

Trichomes as determinants of water repellency

Hairy leaf surfaces, such as those of *Alchemilla vulgaris*, are highly water-repellent because their stiff hairs keep water droplets elevated, preventing contact with the leaf surface and creating a fakir-like effect (Otten and Herminghaus 2004). Brewer et al. (1991) found that plant species with dense trichomes (more than 25 mm^{-2}) tended to be more water-repellent, though some still retained droplets. Similarly, Neinhuis and Barthlott (1997) observed that leaves with wax-covered trichomes were extremely water-repellent, while those with non-waxy trichomes only resisted water briefly after droplet contact.

Inconsistent influence of stomatal density

This observation aligns with the findings of Lott (2021), who reported that stomatal density in five plant species did not significantly influence leaf wettability, possibly due to limited sample size or species-specific traits. All plants in Lott's study had stomata only on the abaxial surface, as is typical in many plant species. However, this finding contrasts with studies by Brewer and Nuñez (2007), who observed that surfaces with higher stomatal densities tended to be less wettable, possibly due to the microstructural complexity that encourages droplet retention. Unlike our findings, Wang et al. (2021) found a positive correlation between stomatal density and contact angle in species studied in Xi'an, China—suggesting that in some cases, higher stomatal density may increase hydrophobicity by contributing to surface microtexture or by trapping air, similar to the Cassie–Baxter wetting regime. Taken together, these contrasting reports suggest that the influence of stomatal density on wettability is context-dependent and may be modulated by other surface features such as cuticular wax, trichomes, and surface roughness. In this study, the lack of a clear trend indicates that stomatal density alone may not be a reliable predictor of hydrophobicity across diverse wetland species.

Surface structure of hydrophilic leaves

Hydrophilic leaf surfaces typically lack the complex micro- and nano-scale structures found on superhydrophobic leaves. They often appear smooth or slightly rough under SEM and show little to no wax crystalloids, which contributes to lower contact angles and increased water spreading (Neinhuis and Barthlott 1997). The cuticle on these leaves may be thin, cracked, or composed of more polar compounds, allowing greater wettability (Riederer and Schreiber 2001). In some cases, visible features such as stomata or trichomes can retain water, further promoting hydrophilic behavior (Brewer and Smith 1997). These surfaces also tend to have higher surface free energy due to the absence of hydrophobic waxes (Koch et al. 2008). As a result, contact angles are typically below 90°, indicating that water readily spreads rather than beads up (Mittal and Davis 2007).The collective absence of epicuticular wax crystals

—a key determinant of hydrophobicity—across all species is a defining trait of these hydrophytes. These traits are not coincidental; rather, they represent evolutionary responses to persistently wet environments where maintaining a thin water layer on the leaf surface is advantageous. Such features may enhance nutrient absorption, support foliar respiration during submersion, and minimize damage from waterlogging (Rascio 2002; Sakamoto and Yamaguchi 2017).

These findings reinforce the view that hydrophilic leaf surface structures are ecologically advantageous in aquatic and semi-aquatic environments. They contrast starkly with the superhydrophobic adaptations found in xerophytic and canopy-dwelling species, where water repellence is vital for self-cleaning and pathogen resistance (Barthlott and Neinhuis 1997; Koch et al. 2009).

Conclusion

This research undertaken examined the relationship between leaf's physicochemical traits and wettability in hydrophytes living in three different ecological conditions i.e. emergent, floating, and submerged. Three plants from each were studied. Contact angle measurements revealed that only three species—*Nelumbo nucifera*, *Colocasia esculenta* (emergent), and *Pistia stratiotes* (floating) exhibited hydrophobic behavior, while the remaining species displayed hydrophilic surfaces. Notably, the two emergent species had aerial leaves that avoid direct water contact, and the hydrophobicity of *Pistia stratiotes* was attributed to the presence of dense, waxy trichomes.

All other plant species showed wetting behavior. Among the various morphological and anatomical leaf traits analyzed, surface free energy (SFE) emerged as the strongest predictor of contact angle. The inverse correlation between SFE and contact angle reinforces the importance of physicochemical properties over purely structural features in determining leaf wettability. All other leaf parameters studied i.e. leaf length, width, area, water adhesion etc. did not have stark impact in determining wettability. SEM analysis further showed that most hydrophilic species lacked micro- and nanoscale hierarchical surface features and epicuticular wax crystalloids—structures typically associated with water repellency in terrestrial species. These findings suggest that leaf wettability in wetland plants is a complex trait shaped by both ecological context and adaptive function. While hydrophobic surfaces in emergent and floating species may aid in self-cleaning and gas exchange, hydrophilic surfaces in submerged or consistently wet leaves may facilitate foliar water uptake, nutrient absorption, or sustained physiological activity under saturated conditions. Thus, wettability patterns reflect functional adaptations aligned with each species' microhabitat and life form. This indicates that leaf wettability is closely linked to habitat-specific ecological pressures and contributes to the functional diversity of plant responses in wet environments. Further research is needed to explore how these traits interact with environmental variables across diverse wetland habitats.

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Author Contribution

All authors contributed significantly to the research and manuscript preparation. STB was responsible for conceptualization, methodology, and experimentation, literature review, drafting the original manuscript. MSN designed the experiments, collected data, and performed formal analysis. TDD conducted the curated data, and contributed to reviewing and editing the manuscript. RVM plant material collection, data validation, critical manuscript review. NVP handled final manuscript editing, proofreading, supervision, and correspondence. All authors reviewed and approved the final version of the manuscript.

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Data availability:

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

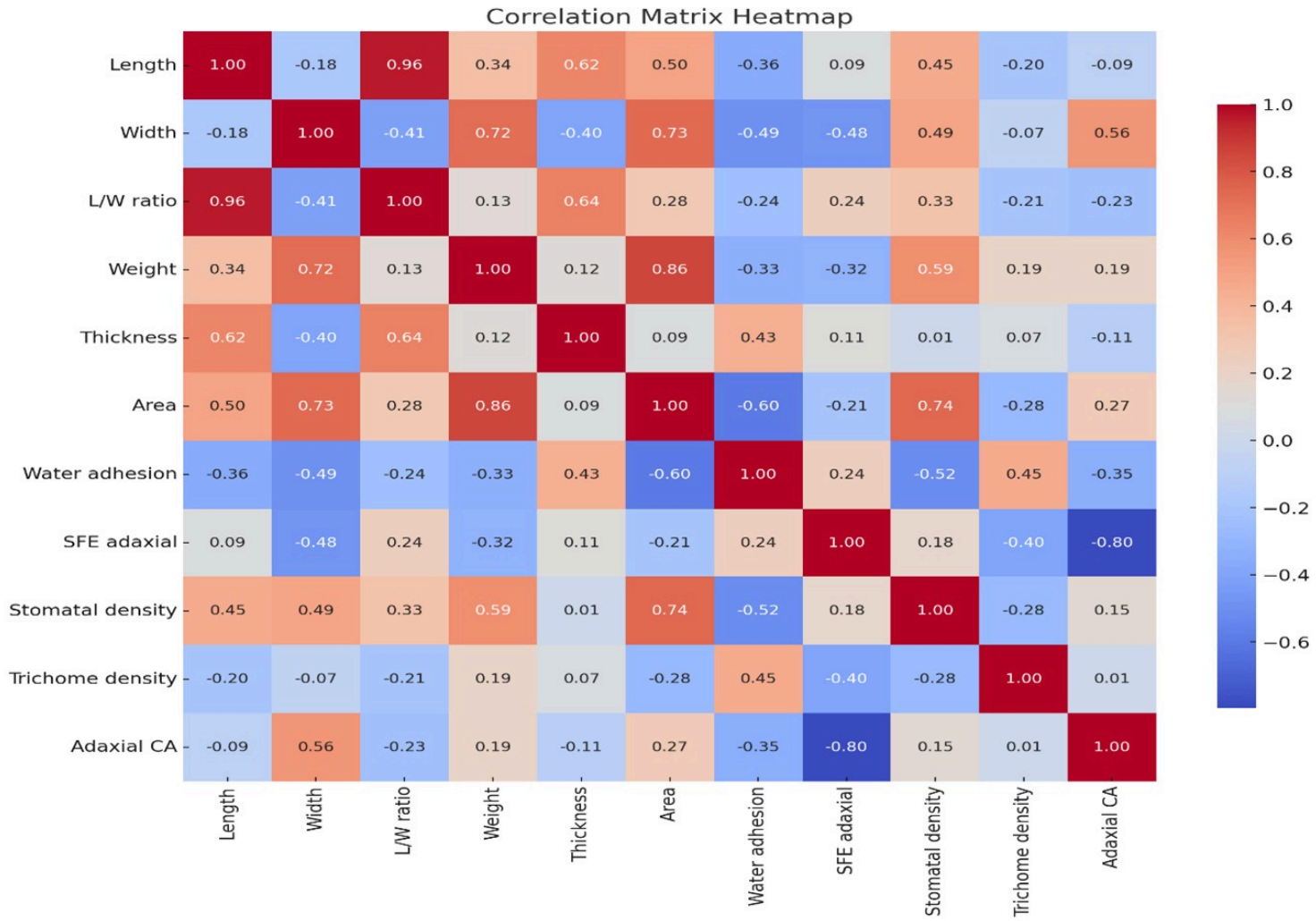

Figure 1

Pearson correlation heatmap showing the correlation coefficient (r) values of every parameter against adaxial contact angles.

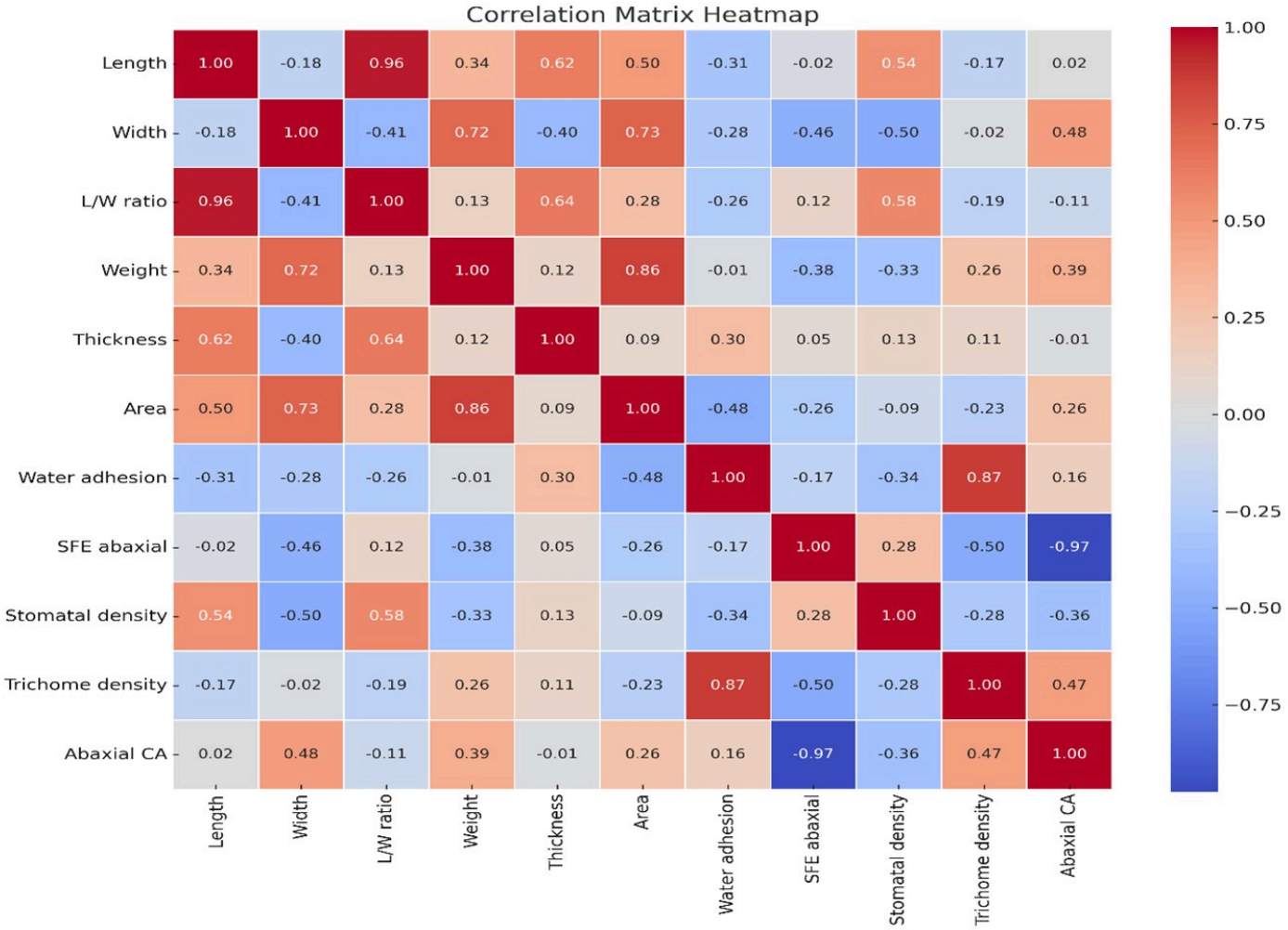

Figure 2

Pearson correlation heatmap showing the correlation coefficient (r) values of every parameter against abaxial contact angles.

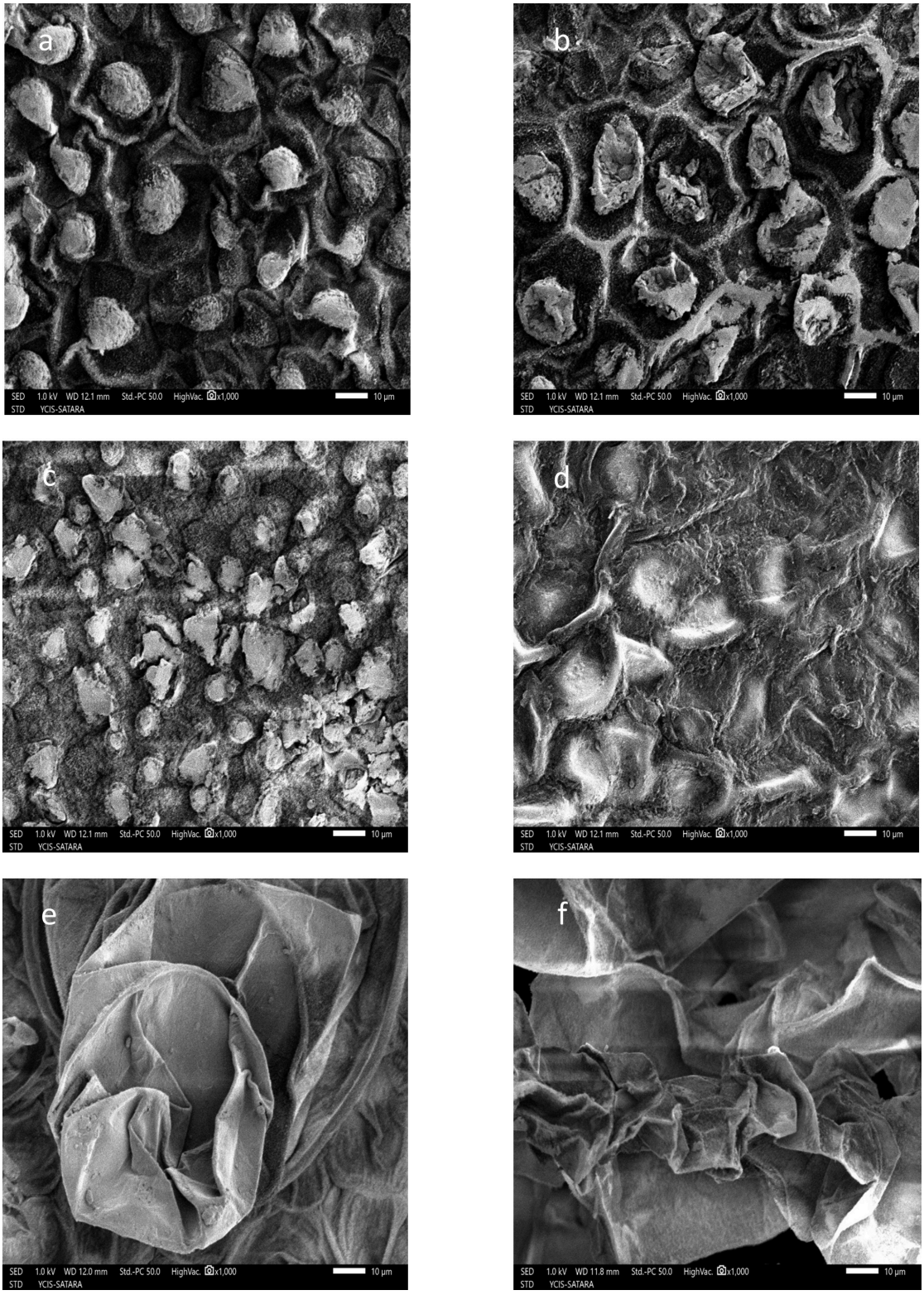

Figure 3

a) b) SEM image of adaxial and abaxial leaf surface respectively of *Colocasia esculenta* showing the hexagonal honey comb like structures. **c) d)** SEM images of adaxial and abaxial leaf surface respectively of *Nelumbo nucifera* showing dome-shaped epidermal cells with wax coating on adaxial surface while abaxial surface has cuticular folds making it less hydrophobic. **e) f)** SEM images of adaxial and abaxial leaf surfaces of *Pistia stratiotes* showing the waxy trichomes which allow water drops to bead up. (All the images are in 1000x magnification).

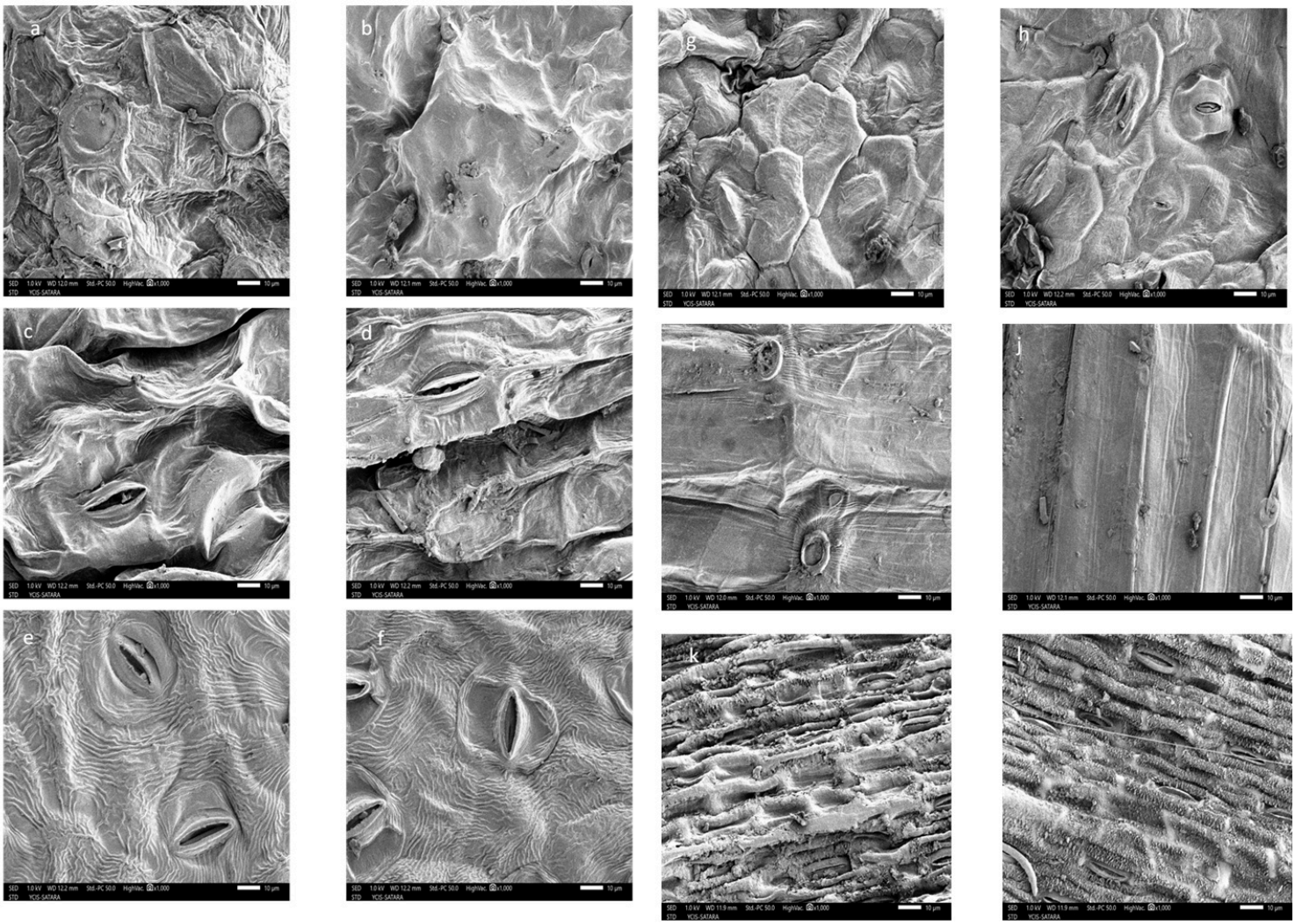

Figure 4

a) b) SEM images of adaxial and abaxial leaf surfaces of *Nymphaea nouchali* showing undulating and irregular epidermis with no micro or nano scaling. **c) d)**SEM images of adaxial and abaxial leaf surfaces of *Cryptocoryne retrospiralis* showing uneven undulating surface, sunken stomata but with no wax crystals. **e) f)** SEM images of adaxial and abaxial leaf surfaces of *Ipomoea aquatica* showing smooth surface with cuticular striations but no wax crystals **g) h)** SEM images of adaxial and abaxial leaf surfaces of *Hygrophila auriculata* with smooth surface and several stomata embedded within shallow depressions and surrounded by smooth cuticular ridges. **i) j)** SEM images of adaxial and abaxial leaf surfaces of Eriocaulon*cuspidatum* showing uniform surface topography with occasional stomatal striations. **k) l)** SEM images of adaxial and abaxial leaf surfaces of *Typha angustifolia* showing uneven and micropatterned structures but lacking wax crystals.