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Research Article

Ecological Significance of Leaf Surface Micromorphology and Wettability in *Tragus berteronianus* Schult

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Abstract

Background and Objective: *Tragus berteronianus* Schult. is an annual grass in dry habitats of southwestern Saudi Arabia. This species reveals dissimilar wettability on both leaf surfaces with dew collecting ability. Therefore, the objective of this study was to determine the micromorphological features of *Tragus berteronianus* Schult, responsible for those characters and their ecological significance. **Materials and Methods:** Both leaf surfaces were studied by SEM. Dimensions of surface microstructures were measured and analyzed. Elemental analysis of the leaf epidermal surface was determined by energy-dispersive X-ray spectroscopy (EDS). Dew collecting ability was observed in the field as well as in the laboratory by exposing leaf surfaces to the mist stream generated by a cold mist humidifier. Contact angles of droplets precipitated on the leaf surfaces were measured from digital images. **Results:** Adaxial leaf surface showed hierarchical structures from sub-millimetric to micro- and nano-scale, in which dimensions of micro projections and spacing between them denoting to Cassie state. Abaxial leaf surface, in contrast, showed a different pattern of microstructures, in which dimensions of micro projections and spacing between them denoting to Wenzel state. The adaxial leaf surface was superhydrophobic, whereas the abaxial surface was hydrophilic. Spines of leaf margin have a high ability to collect dew. **Conclusion:** Wettability in *Tragus berteronianus* leaf differs on both leaf surfaces due to microstructure traits and can explain high adaptation in dry habitats by using dew efficiently as an alternative source of water.

Key words: *Tragus berteronianus*, microstructures, wettability, superhydrophobic, hydrophilic, adaptation, dry habitats

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Photosynthetic surfaces of the shoot have often been considered the most morphologically and anatomically variable organ of the plants[1]. Leaves, among them, are the common part in this context. Because plants are sessile organisms, leaf surfaces in them play crucial roles in environmental interactions[2]. Epidermal cells represent the surface layer of leaves, consisting of a cuticle in the outermost part. The cuticle comprises two lipid components, cutin and waxes, in which waxes embedded in the cutin matrix and some are deposited on the surface of the cuticle and called epicuticular wax[3,4]. Epicuticular waxes that cover cuticles are variable in thickness and micromorphology[5]. The Cuticle is responsible for giving the leaf surface many of its characteristics and micromorphological structures[6,7]. Although the main function of cuticle in most land plants is the reduction of water loss, the diversity of leaf surface structures is pointing to other functions or even multifunction in the same plant[2,7,8]. Most of the functions of leaf surfaces arise from a variety of micro-scale structures[2,8].

One of the main characteristics of leaf surfaces is wettability. Wettability describes to any extent the liquid comes in contact with a solid surface[9].

Leaf wettability represents an important plant functional trait with critical roles in environmental interactions[2,9]. Leaf Surfaces of plants are greatly variable in wettability, from hydrophilic (wettable) to superhydrophobic (highly non-wettable)[10-14]. The degree of wettability depends on cuticle physicochemical properties, which represented by the chemical composition of cuticle wax and surface micromorphology. Although the basic nature of the cuticle is lipid materials, leaf surfaces of smooth wax film or with isolated wax micro projections are hydrophilic. The presence of trichomes without wax micro projections also makes the surface hydrophilic[7]. On the other hand, most leaf surfaces have hydrophobic waxy nature with or without dense wax micro projections (3-D epicuticular waxes). It is known that the roughness of a hydrophilic surface increases wettability, while the roughness of a hydrophobic surface increases its water-repellency[6,15]. As surface free energy increased by increasing surface area according to intermolecular forces of surface material, hydrophilic surfaces have high surface free energy that formed by polar molecules comparing with hydrophobic surfaces that have low surface free energy due to non-polar molecules[10,16].

In literature published until now, there is a rough estimation of plant species according to leaf surfaces wettability, in which hydrophobic surfaces are predominantly comparing with hydrophilic surfaces[2,7,17,18]. The predominance of hydrophobic plant surfaces may largely be based on the hydrophobic nature of the waxy surface of cuticle[7]. Although, some studies revealed that among plant species and both adaxial and abaxial leaf surfaces, there is high diversity in wettability[11,14] which depends on the fact that wettability arises from both physicochemical properties, not only chemical nature of the surface.

Many studies about wettability and its phenomena for plant leaves were concentrated either on dew or fog harvesting ability by special surface microstructures[19-24], or hydrophobic surfaces, especially aquatic and humid region plants[12,17,25-28], of which lotus leaves (*Nelumbo nucifera*) represent famous example and led to a phenomenon called "lotus effect" denotes to superhydrophobicity with self-cleaning surfaces[12]. On the other hand, far less attention has been paid to the plants of arid or semiarid habitats in this context[14,17].

Carrot-seed grass *Tragus berteronianus* Schult. (Poaceae) is a small annual plant, native to many parts of warm Africa and Eurasia and has been naturalized in the warm regions of Americas[29,30]. In Saudi Arabia, this species grows in open dry habitats in the Southwestern region. The plant has a prostrate growth habit, with culms up to 10 cm tall.

During a field trip to arid habitats east of Tihama, Jazan region, Southwestern Saudi Arabia (January 2020), the author notices that *Tragus berteronianus* have dense dew droplets condensed on the leaves in the early morning. The dew droplets were nearly in perfect spherical shapes, indicating to superhydrophobicity of leaf surfaces.

This study aimed to investigate the reasons behind this phenomenon and its ecological role during the short life cycle of this annual plant.

MATERIALS AND METHODS

Study site: The study was conducted in the dry open area, NE of Sabya, about100 km NE of Jazan city, 17°19'N, 42° 48'E. The climate characterized by a high temperature of 30°C as an annual mean and low precipitation rate of ~150 mm/year. The rainfall season is mainly in the summer months. Vegetation is sparse with patches of xerophytic shrubs, dominated by *Acacia tortilis* and some succulents. Annual plants thrive for a short time after the summer rain, with low diversity, dominated by few grasses species, of which *Tragus berteronianus* represent one of the most prominent annual species.

Field observations and samples collection: Three field trips were conducted during January 2020. Field observations were performed in the early morning. Imaging of dew droplets was made by a macro lens (Tamron SP 150-600) and Nikon D300s camera. Leaf samples were collected and dividing into two sets, one stored in 70% ethanol to subsequent examination by scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS), whereas other transferred freshly to the lab for airborne moisture collecting experiment by spines.

SEM microscopy and EDS analysis: Dried pieces of leaves (about 5×3 mm) were mounted on the stub on double side carbon tap, sputter-coated with gold and examined under high vacuum with an accelerating voltage of 10 kV by SEM (JSM-6380 LA-JEOL, Japan). Measurements of leaf surfaces microstructures were made directly from SEM images. Elemental analysis of leaf epidermal surface was determined by energy-dispersive X-ray spectroscopy (EDS) (JSM-6380 LA-JEOL, Japan).

Airborne moisture collecting experiment: The dew collecting ability of leaf surfaces was observed in the field. As well as droplets that seen on the leaf surfaces, spines of leaf margins, revealed a noticeable dew collecting ability. It is very difficult to mimic dew formation conditions in the laboratory due to many reasons, of which lack of radiative cooling to reach the dew point temperature in the right climatic conditions[31,32]. On the other hand, cone shape structures (like spines) have the same principle to collect airborne moisture both in cases of fog, that can be mimic by mist humidifier, or dew in natural conditions, unlike other surfaces[31,33]. To reveal spines ability to collect airborne moisture in the laboratory, fresh and clean leaves were chosen (5-7mm length), fixed in glass slides in room temperature and then put horizontally in front of purifying mist stream generated by a cold mist humidifier (BLACK+DECKER HM3000), at a distance of about15 cm from the mist outlet. The flow of the mist was adjusted by control dial to low flow rate of ~1 mL h^{-1}. The speed flow of the mist stream was ~0.7 m sec^{-1}. This low flow rate was chosen to mimic normal conditions as possible and then reveal the efficiency of airborne moisture collecting with more precisely. Airborne moisture collection ability of spines was performed for 1 min. and immediately imaging by digital camera adjusted to a stereomicroscope (SONY FD Mavica 2.0 MP).

Contact angle measurements of dew droplets: Side view images of the droplets were captured in the field and laboratory. Digital images of droplets were used for contact angle measurements by image processing software ImageJ [34]. The same software was also used for the measurement of the apex angle of the spines (δ).

Statistical analysis: All measurement and analysis results were prepared with at least three replicates. Statistical analysis was performed by student's t-test ($p<0.001$).

RESULTS

Micromorphology of leaf surface and wettability: Enlarged adaxial leaf surface shows a prominent microstructure comparing with the abaxial surface (Fig. 1). Leaf surface, as many grasses, divided into longitudinal zones of sub-millimetric ridges and grooves between them (Fig. 1a). In adaxial surface, these ridges and grooves not variable in deeping or spaces between them, while in the abaxial surface more variable in deeping and spaces between them was observed (Fig. 1b and c). The mean wide of the groove in adaxial surface was 101.2±7.7 µm, while wide of the groove in abaxial surface was 16.6±6.6 µm. More magnification of adaxial leaf surface revealed vast number of microstructure projections (micropapillae) and epicuticular wax bumps (at nano-scales) covering all the surfaces (Fig. 1d). Abaxial leaf surface revealed many silica bodies covering the surface (on the ridges) with apparently sparse wax bumps especially between silica bodies (Fig. 1c). Dimensions of both micro projections (papillae and silica bodies) were variable on the two surfaces (Table 1). The mean diameter and high of every papilla were 6.5 and 6.4 µm, respectively, with spacing between them of 8.5 µm. On the other hand, mean diameter and high of every silica body were 12.8 and 2.1 µm, respectively, with spacing between them of 7.5 µm. The ratio of high-to-space (h/s) of micro projections were 0.75 and 0.28 for adaxial and abaxial, respectively. Papillae on the adaxial surface were more density comparing with silica bodies on the abaxial surface, with values of 3575 and 1365 per mm^2, respectively.

In the early morning (5-7 am), spherical droplets of dew were observed on the adaxial leaf surfaces (Fig. 2a). These

Table 1: Microstructure characters in both leaf surfaces of *Tragus berteronianus*

	Microstructure projections					
Leaf surface	d (µm)	h (µm)	s (µm)	h/s	Density (N/mm^2)	Contact Angle (CA°)
Adaxial	6.5±0.7	6.4±1.2	8.5±2.8	0.75	3575±156*	155.0°±4.5*
Abaxial	12.8±1.0	2.1±0.5	7.5±6.4	0.28	1365±108.9	88.9°±20

d: Diameter of the microstructure projection (micropapilla in the adaxial surface and silica body in the abaxial surface), h: High of the microstructure projection, s: Mean space between every two microstructure projections, *Significance at $p<0.001$

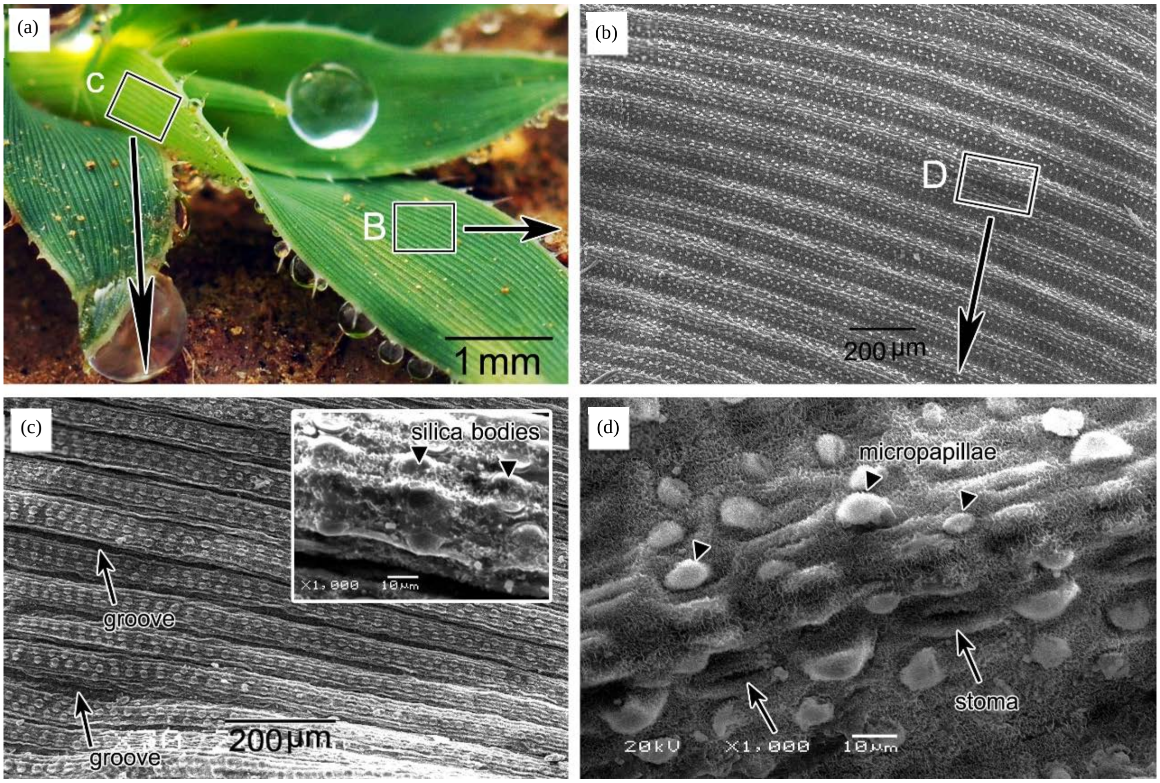


Fig. 1(a-d): (a) Leaf surfaces of *Tragus berteronianus*, (b and d) SEM images of adaxial surface, (c) SEM image of abaxial surface

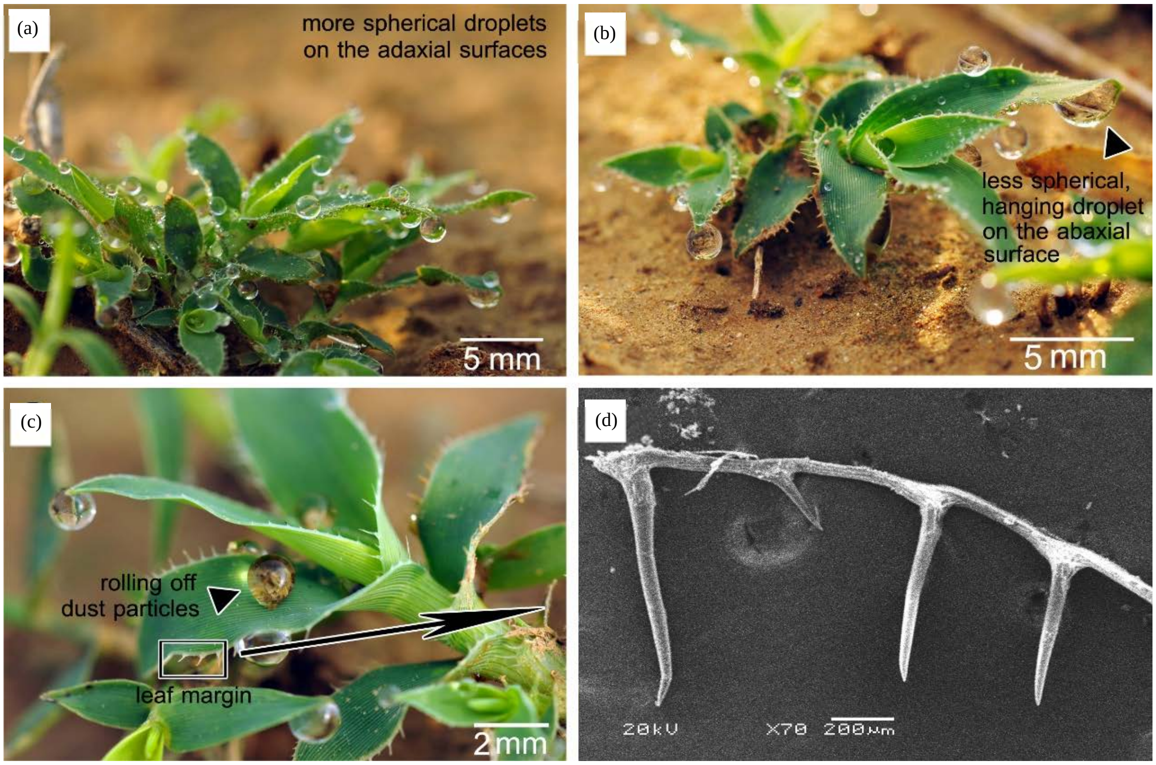


Fig. 2(a-d): (a-b) *Tragus berteronianus* leaves in its natural habitat at 7 am, (c-d) Leaf margin with pectinate spines

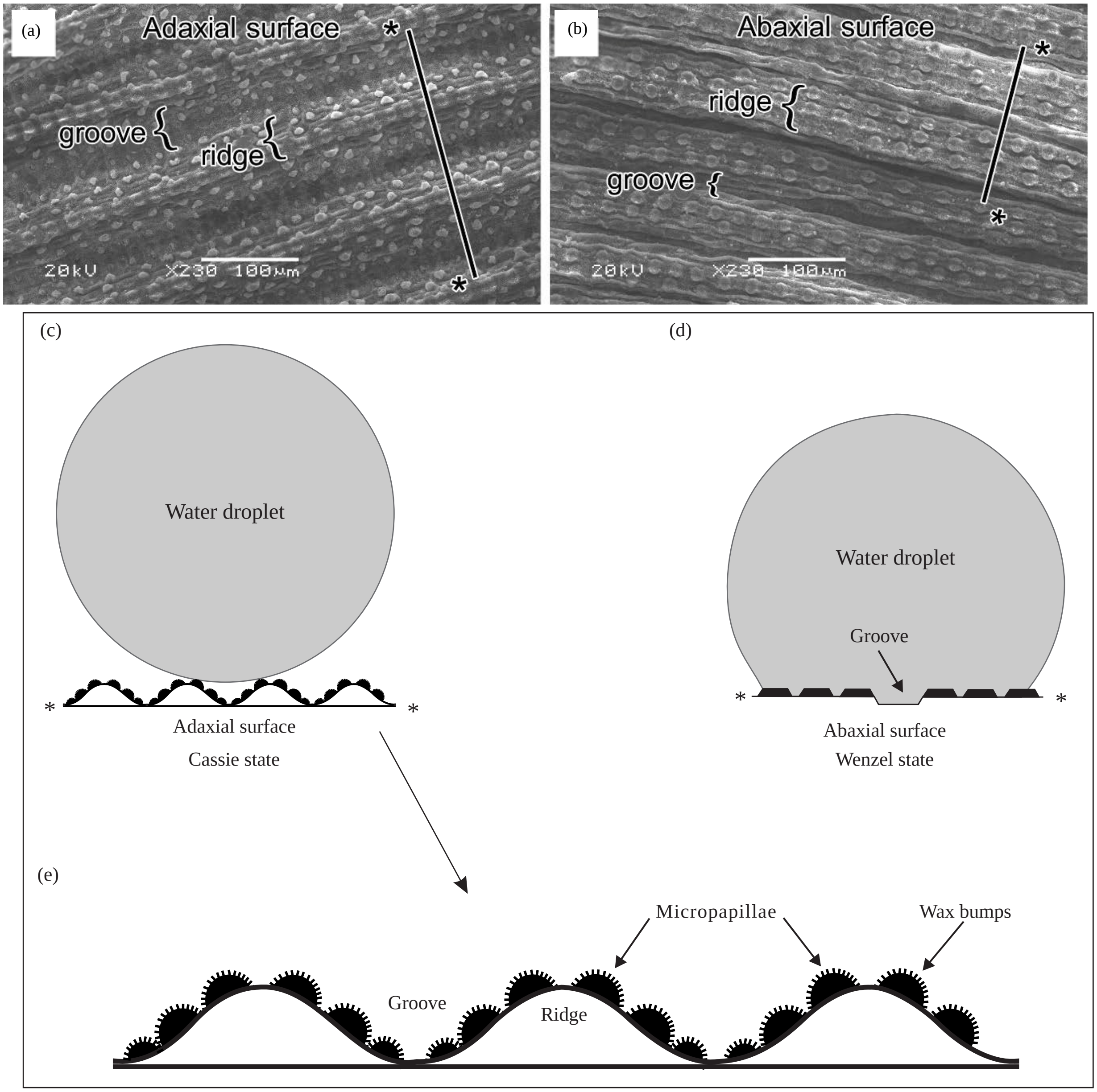


Fig. 3: The hierarchical structures of leaf surfaces with droplets behavior (Cassie state and Wenzel state), (a and b) Sub-millimetric scale (grooves and ridges), (c) Adaxial leaf surfaces, (d) Abaxial leaf surfaces, (e) Micro- and nano scale (micropapillae and wax bumps)

droplets were rolled down and fall directly to the near soil surface. On the abaxial surfaces, the droplets were less spherical and not easy to fall but exhibit high adhesion (Fig. 2b). Contact angles of droplets on both adaxial and abaxial surfaces were 155 and 88.9°, respectively.

Leaf margin have pectinate spines (Fig. 2c and d) and making a hyaline-tough frame around the leaf. Mean length of the spine was 740±266.5 μm, with a mean width of 45.25 ±10.8 μm. Apex angle (δ) of the spine was 28.1±1.1°.

Characteristics of leaf micromorphology with its structures on both surfaces show a hierarchical manner from sub-millimetric (grooves and ridges) to micro- and nano-scale (micropapillae and wax bumps, respectively), which are connected with wettability on both surfaces (Fig. 3a-e). Spherical droplets of dew were observed on the adaxial leaf surfaces, while less spherical droplets were observed hanging on the abaxial surfaces (Fig. 3c and d).

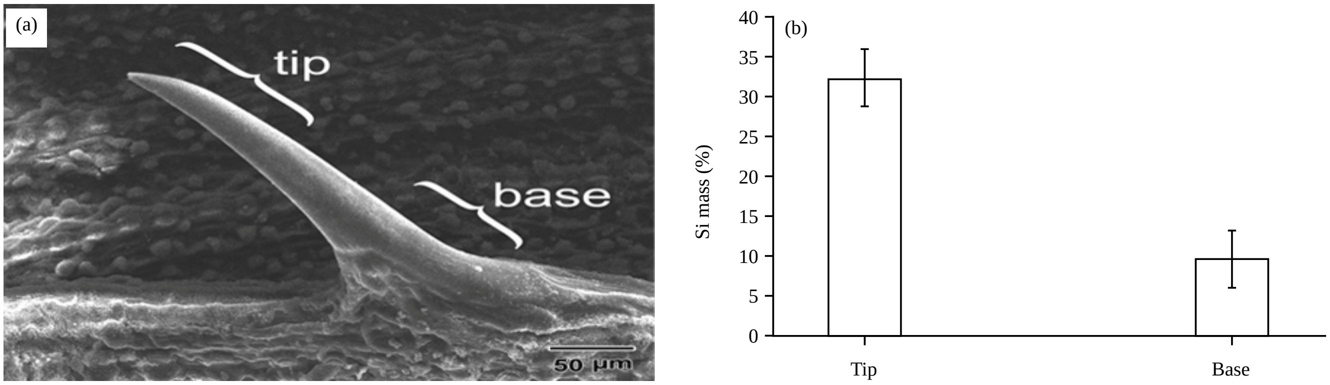
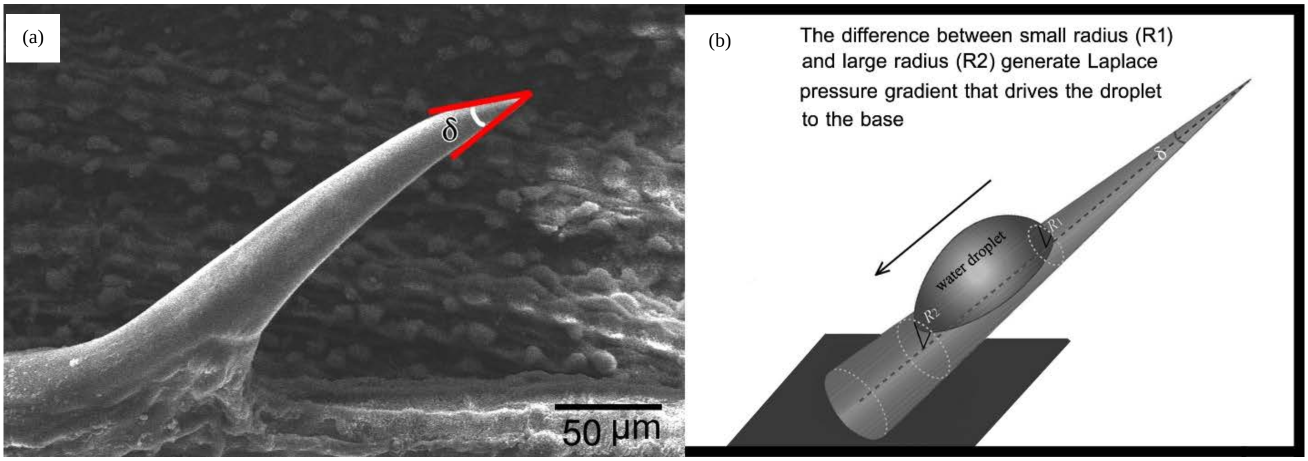


Fig. 4(a-b): (a) SEM image of margin spine, (b) Silicon mass percentages of the two spine's parts



The difference between small radius (R1) and large radius (R2) generate Laplace pressure gradient that drives the droplet to the base

Fig. 5(a-b): (a) SEM image of a single spine reveals apex angle (δ) which is = 28°, (b) Geometry of spine (cone shape)

Table 2: Silicon percentages and carbon/oxygen molar ratio in some parts of *Tragus berteronianus* leaf

Leaf trait	Si (mass percentage %)	C/O molar ratio
Spine		
Tip	32.400±3.5	1.4
Base	9.500±3.2	1.4
Hyaline frame	0.70±0.1	1.6
Silica bodies	20.8±1.6	1.0

Elemental composition analysis of the surface material of spines indicated of high silicon (Si) content on the basis of mass percentage. Silicon mineralization patterns were variable in regions of spine, in which tip regions were having more silicon than base regions. (Table 2, Fig. 4). On the other hand, the hyaline frame has a very low content of Si with carbon to oxygen molar ratio of 1.6 (Table 2). The surface of silica bodies (that have almost non-waxy deposits) have 20.8 Si as a mass percentage. Spines with its characters seem to play a role in wettability and moisture collecting, as observed in both field and laboratory (Fig. 5 and 6).

Dew condensation experiment: After 1 min of exposed to mist stream of cold mist humidifier in the laboratory, leaf surface located in front of mist stream showed deposited small droplets on the leaf surfaces, in a manner near that in the field (but with small sizes, 200-500 µm in diameter, compared with 1-3 mm in the field). Spines show a prominent ability for moisture collecting. Droplets appeared to start at the tips of spines, growing gradually and coalesce together to bigger droplets, which move spontaneously to the base (Fig. 6a-c). After moving to the base, droplets appear to absorbed consecutively inside the hyaline frame. Duration for the whole

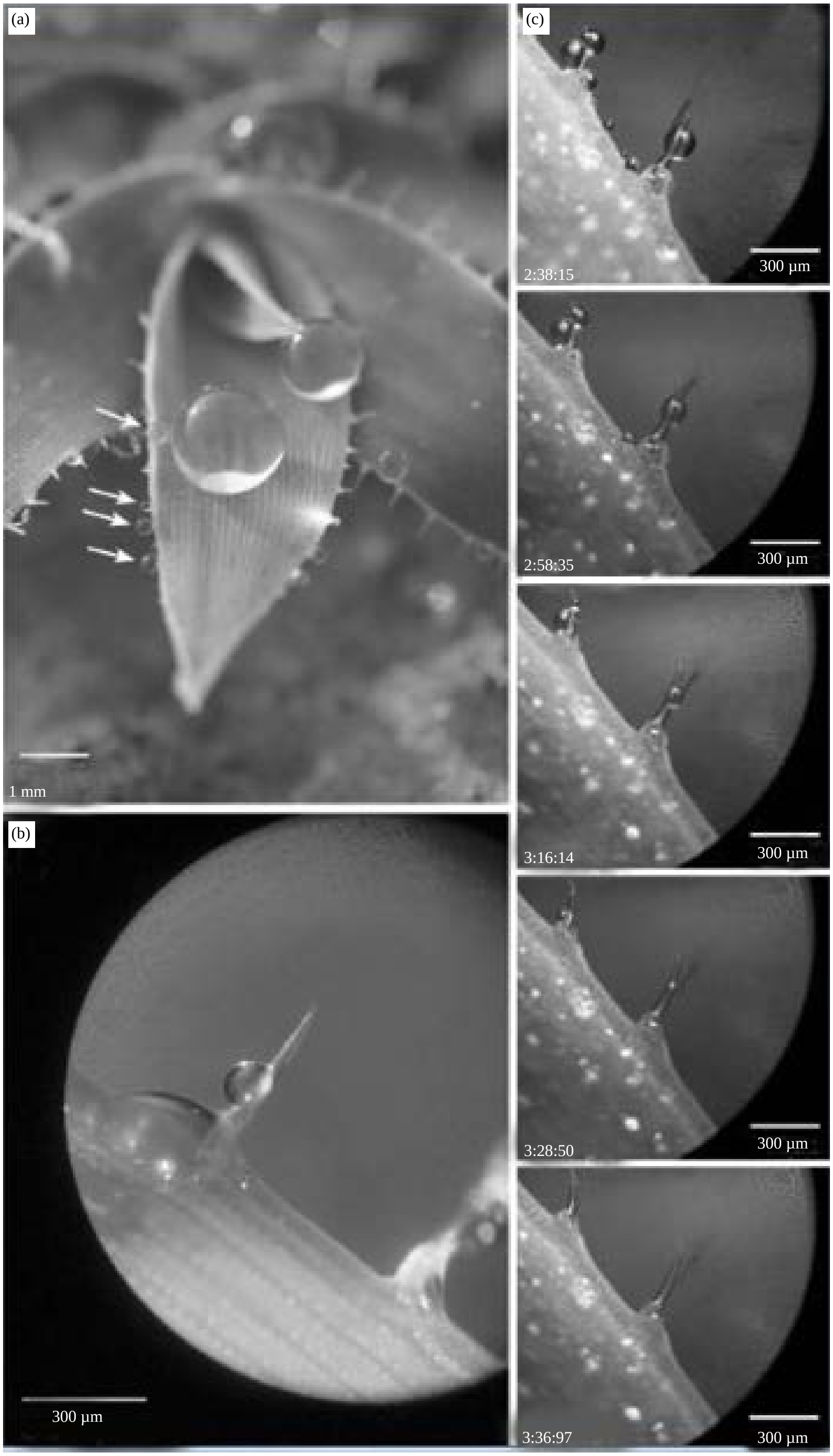


Fig. 6(a-c): (a) Dew droplets on the adaxial leaf surface in the field, (b-c) Time-lapse of droplets behavior on the spines (~58 sec) process of dew collecting by spines and absorbing at the base was ~50-60 sec. In the field, the dew collecting ability of spines was clear but with less absorbing in the base region in the end of the dew formation period.

DISCUSSION

It has been evidenced that the wettability of the leaf surface is governed by both surface micromorphology and chemical nature[8,35]. Nevertheless, compared with the surface micromorphology, the impact of the chemical nature of the surface on the wettability is relatively smaller[36]. In the case of surfaces with micromorphological structures, wettability can be explained according to two distinct classical models, Cassie state[37] or Wenzel state[38]. Cassie state characterizes the condition where a droplet rests on the surface without permeation between microstructure projections, in which air instead of water is trapped between microstructure projections, increasing hydrophobicity. Wenzel state, on the other hand, describes the condition where a water droplet penetrates the spaces in between microstructure projections of the surface, resulting in fully wets of the contact area of the surface without air-pockets between microstructure projections (droplet shows more adhering to surface). Adaxial leaf surface in *Tragus berteronianus* revealed hierarchical structures of levels from sub-millimetric (ridges and grooves) to micro- and nano-scale structures (micropapillae and wax bumps on them, respectively). The dimensions of micropapillae and spacing between them, with ratio of height to the spacing of 0.75, denote to Cassie state in which air trapped between microstructures (especially micropapillae and wax bumps)[36,39] (Fig. 3c). Cassie state characteristic of adaxial surface reflected also by high contact angle (>150°) which indicated to superhydrophobicity[10]. On the other hand, the longitudinal arrangement of ridges and grooves along leaf surface lead to an anisotropic flow of any droplets deposited on the surface (flow easily along longitudinal directions than orthogonal directions), which is resembling that of rice leaf[40]. These characteristics of adaxial surface lead to increase water repellency in which droplets rapidly gain momentum and therefore easily roll off and fall to the ground. Superhydrophobicity of adaxial surface in *Tragus berteronianus* leaves provide self-cleaning properties by the above characteristics, as in lotus "lotus effect"[9,12]. This trait of the surface makes it easy to pick up any contaminating particles (dusts or so) by water droplets and carry away during roll off and fall to the ground, leaving the surface always clean after every precipitation event. Keeping the photosynthetic surface clean is very important to enhance photosynthesis rate, as contaminating particles may plug stomata as well as reduce receiving photosynthetically active radiation[41]. Furthermore, as a plant in a prostrate growth habit, the water droplets on such surfaces with easily rolling off, fall directly on the soil beneath leaf surfaces, increasing moistening of upper soil surface (which was observed in the field) giving additional water source to the shallow roots in this small annual plant during its short life (~60 days). Although wetting of leaf surface reported to have positive effects on plant function like reducing transpiration rate and enhance of water use efficiency[42,43], negative effects can lead to some damage in leaves. Wetting of leaf surfaces can reduce carbon assimilation rate, as CO_2 diffusion is about 10,000 times more slowly in water than air[44,45]. Furthermore, the persistence of water droplets on leaf surfaces can cause sunburn as a consequence of intense focusing sunlight effect[46].

Abaxial leaf surface in *Tragus berteronianus* revealed silica bodies covering the surface with the less prominent manner and low density compared with the adaxial surface, as well as sparse wax bumps. The ratio of high-to-space (h/s) of micro projections (silica bodies) was 0.28. These characters are denoting to Wenzel state in which a water droplet penetrates into the specs in between microstructure projections of the surface, resulting in fully wets of the contact area of the surface without air-pockets between microstructure projections[10,36] (Fig. 3d), exhibiting high adhesion and low contact angle for hanging water droplets. As stomata not observed in the abaxial leaf surfaces and these surfaces not receive photosynthetically active radiation or contaminating particles like adaxial surfaces, wetting of this side of *Tragus berteronianus* leaf may enhance of water use efficiency[42,43]. Silica bodies in the abaxial surface enhance the mechanical strength of leaf with low energy costs compared to lignification, in which energy costs of silicon deposition were estimated to be 20-times lesser than normal lignification[47]. This character may enhance economize in metabolic energy to spend it to other anabolic activities.

Spines of the leaf margin seem to play an important role in capturing and driving dew droplets, as observed in the field and laboratory. Spines are not covered by wax, making them hydrophilic, enhancing the wettability of these surfaces[7]. The spines have a conical shape with a value of cone-apex angle (δ) of 28.0°. The conical shape of such geometrical features produces a Laplace pressure gradient[24,48]. The tip of the cone (tip of the spine) has a larger Laplace pressure than the base of the cone (base of the spine). This difference generated from the small radius-high curvature at the tip of the spine to the large radius-low curvature at the base of the spine (Fig. 5b). The Laplace pressure gradient along the spine represents the driving force that leads to spontaneous movement of the droplet from the tip of the spine to the base[20,24] (Fig. 5b). Under favorable conditions, water molecules tend to captured as a very small droplet on the tip of the cone structure (like spines)[20,49]. With continuous deposition, small droplets coalesce with each other to big droplets in the way to the base and eventually absorbed at the area of the base as seen in the laboratory.

Elemental composition analysis of the surface material of spines indicated high silicon (Si) content, especially in the tip regions. This pattern of Silicon mineralization reinforces stiffness of the spine's tip, leading to enhancement of dew capturing. On the other hand, spine base and the hyaline frame has a very low content of Si with carbon to oxygen molar ratio of 1.6, an indication to cellulosic materials[50]. The predominance of cellulosic materials in the spine base can explain the absorption of droplets in this part. Such foliar absorption of dew was reported in some arid and semi-arid plants[51,52]. At the end of the dew formation period (in the early morning) in the field, hydration of leaves seems to be in a saturated state, which is may explain less absorption of dew droplets as seen in persistent droplets on the spine base and hyaline frame. This behavior of leaf wettability in *Tragus berteronianus* due to surface micromorphology may explain the survival of this annual species in its dry habitats by using dew as an alternative source of water to maintain leaf turgor and carbon assimilation efficiently for complete its life cycle.

CONCLUSION

Based on present study results, it can be concluded that *Tragus berteronianus* have remarkable micromorphological characters of leaf surfaces, enable them of having contrasting wettability. The adaxial surface is superhydrophobic with self-cleaning properties, while the abaxial surface has hydrophilic properties. Such characteristics demonstrated for the first time in this species and can explain high adaptation in dry habitats by using dew efficiently as an alternative source of water, enhance soil surface moisture and maintaining positive water status and carbon assimilation during the plant life cycle.

SIGNIFICANCE STATEMENT

This study revealed that the contrasting wettability of leaf surfaces in *Tragus berteronianus* was caused by micromorphology from sub-millimetric to micro- and nano-scale structures. The surface micromorphology of leaves is greatly affected wettability properties according to the shape and dimensions of microstructures, with some ecological roles. The results of this study suggested that microstructures of leaf surfaces in *Tragus berteronianus* enhance the adaptability of plants with dry habitat conditions. On the other hand, such characteristics of leaf surfaces can act as templates for biomimetic artificial materials with different wetting features.

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