Ultrastructural studies on tendrils of plant climbers reveal a hierarchical tissue organization: A microscopic investigation

Narasimhan S*
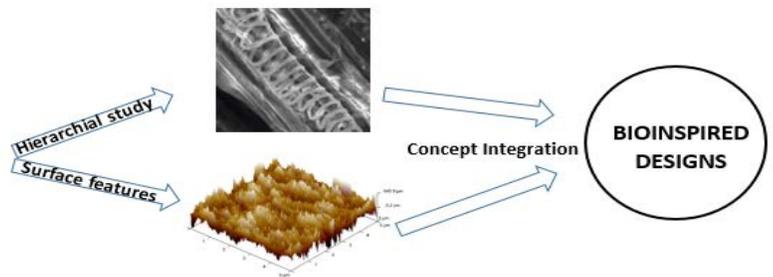
Department of Biotechnology, Manipal Institute of Technology, Manipal Academy of Higher Education, Manipal, Karnataka, India.

Article

ABSTRACT

Tendrils are natural morphological designs that function as climbing adaptations adopted by weak-stemmed plants to attach over strong-stemmed supporting hosts or different artificial support systems. Tendrils are frequently either a modified stem, axillary bud or a modified leaf. Tendril recognises its ideal support hosts with its coiled spring onto which it finally attaches. Robotic engineers often quest for upgraded structures that can sense and grip. The present study focuses on comprehending the underlying structural features naturally present in plant tendrils. The study highlights three different types of plant tendrils. Evaluation of plant tendril morphometry is done by various high-performance structural analytical tools, notably Scanning Electron Microscopy, Atomic Force Microscopy and Fluorescence Microscopy. The ultrastructural features revealed the unique architectural design of cellulose fibrils and tissue-level organization in the anatomy, which enables the mechanical function of the tendrils. Additionally, the study supports that there are six different hierarchical chirality length scales. To improve and redesign machines, notably in robotics; to endow them with sensory perception, an analysis of the microscopic cellular structure of tendrils may be beneficial. The study also emphasizes that an extraordinary design with clear hierarchical patterns is required rather than replicating the shape.

INTRODUCTION

Climbing plants are an impressive category of kingdom Plantae when we consider the bioengineering aspects.[1,2] The mechanisms, as well as the structures used by these climbing groups, are highly remarkable, especially for sensory modelling in machines. Climbing plants exhibit remarkable mechanisms and structures that have the potential to inspire the development of sensory modelling in machines.[1] These botanical wonders showcase a remarkable synergy of biology and engineering principles, offering valuable insights into creating efficient, adaptive, and versatile sensory systems for artificial intelligence and robotics. One of the most striking features of climbing plants is their ability to sense and respond to their environment.[3] They employ a diverse array of sensory mechanisms, such as photoreceptors to detect light, gravitropism to sense gravity, and thigmotropism to perceive physical contact. These sensory systems enable them to navigate complex terrain, find support structures, and optimize their growth. This sensory diversity is akin to the sensory modalities employed in robotics, where vision, touch, and other senses are essential for intelligent decision-making. The structures utilised by climbing plants are equally fascinating. Twining plants like the morning glory employ helical growth and spiral tendrils to latch onto supports, demonstrating innovative mechanical designs.[4] This concept could inform the development of adaptive robotic appendages that mimic these natural tendrils, providing robots with the ability to grip and interact with their surroundings more effectively.[4,5]

*Narasimhan S. Department of Biotechnology, Manipal Institute of Technology, Manipal Academy of Higher Education, Manipal, Karnataka 576104 India
Tel: 91-0820-292224326
Email:narasimhan.s@manipal.edu

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The purpose of climbing adaptation in weak stemmed plants is towards better capture of sunlight as well as for pollination and seed dispersal. Botanically climbing plants can be classified as (i) Root climbers (ii) Stem climbers (iii) Tendril climbers (iv) Hook climbers (v) Scramblers and (vi) Lianas. Among these, tendrils in climbers are unique because of the presence of a natural "spring" which can be a modification of either a leaf or an axillary bud.[3] Multiple factors are involved in tendril mechanisms of plants such as (i) biomechanics (ii) coiling (iii) non-linear dynamics and (iv) morphometric characters.[4,5] Because of these naturally occurring engineering aspects, tendril bioinspiration ideas have been adopted in robotics,[6] ultra-stretchable integrated electronics[7] and miscellaneous fabrications.[8]

Since plants are sedentary forms, they possess diverse morphological adaptations, that aid them to thrive in diverse habits and habitats. The present study discusses tendril of three different plants viz., (1) *Benincasa hispida*, a member of the plant family Cucurbitaceae with a branched tendril modified from an axillary shoot[9] (2) *Passiflora edulis,* a climber belonging to the family Passifloraceae, where the axillary bud is modified into an unbranched tendril and (3) *Gloriosa superba* in which the leaf tip is modified into a tendril. According to the findings, the chosen climbing plants are worth modelling in machine design. Their ability to adapt to changing situations provides an exciting paradigm for self-optimizing and energy-efficient robots.

EXPERIMENTAL

Plant specimens collected for understanding tendril mechanism were., (1) *Benincasa hispida* (2) *Passiflora edulis* and (3) *Gloriosa superba.* The plant specimens were first identified at the Department of Biotechnology, Manipal Institute of Technology, Manipal, Karnataka, India and voucher specimens were deposited in the herbarium for further reference.

Light microscopy analysis was performed by taking freehand sections. These sections were observed in visible light as well as with UV illumination under an Olympus BX51 microscope. For revealing the ultrastructural characters of the tendril, Scanning Electron Microscope (SEM) analysis was used. SEM analysis was conducted by a Carl Zeiss EVO 18 Special Edition SEM with a low voltage of Electron High Tension of 15.00 kV. Image processing and analysis were done using the SmartSEM version 5.05 software available with this instrument. SEM analysis was carried out for revealing the tendril surface architecture. To study the internal tissue ultrastrctural architecture, freehand sections were observed under SEM. Atomic Force Microscopy (AFM) was used to review the minuscule 3-D surface design and architecture of the tendril.

AFM analysis was conducted by using Bruker Innova AFM equipped with NanoDrive 8.02 software. Studies were made in the tapping mode with silicon tapping-mode- tips. The scan range was 5 μm X 5 μm. Other parameters, such as the spring constant, were calculated by following Hook's law and the diameter of the tendrils was measured by using a screw gauge.

RESULTS AND DISCUSSION

The average length of the tendril taken for study (7 replications) was 15.5 cm in *B. hispida*, 1.20 cm in *P. edulis* and 0.3 cm in *G.*

superba. In *B. hispida* tendrils were branched (Figure 1a). The number of coils varied from 20-30 in *B. hispida* (Figure 1a),10-15 in *P. edulis* (Figure 1b), and 3-4 in *G. superba* (Figure 1c, d)*.* In *B. hispida,* tendril thickness was 3 mm at the base, 1 mm at the middle and 0.35 mm towards the tip. While in *P. edulis* it was 1.5 mm at the base, 1 mm towards the middle and 0.85 mm towards the tip. In *G. superba* it showed a diameter of 0.3 mm towards the origin and 0.2 mm towards the tip. It was found that no contact is required for coiling (Figure1b). Tendrils were able to hold on each other (Figure 1b) and were able to serve the function even after turning necrotic and drying.

Figure 1: Tendrils analysed for the study (a): *B. hispida* (b): *P. edulis*. Note the tendrils are able to attach each other (c): *G. superba* habitat (d): the leaf tendril

Tendrils in *B. hispida* possessed several trichomes on it (Figure 2a). However, no stomata were present. The tendril of *P. edulis* owned closely adhered coils (Figure 2b). Another characteristic feature in this plant was the occurrence of several stomata. The tendril cells of *B. hispida* are broader than that of *P. edulis.* The length of the cells ranged from 60 μm to 240 μm, while in *P. edulis* it was from 1500μm to 300μm. In both cases, the cells appeared broader in the middle and narrower towards the end (Figure 2c and 2d). Epidermal cells of *P. edulis* were spindle-shaped (Figure 2d). The arrangement of the cells was similar to bricks on a wall.

The terminal part of the tendril revealed a vivid morphology, and in this study, each tendril was found to be plant specific. In the case of *B. hispida,* the terminal part appeared smooth towards the tip (Figure 2e), while in *P. edulis* there was no difference in the cellular arrangement and appearance compared to the bottom portions of the tendril (Figure 2f). In the leaf tendrils of *G. superba*, a peculiar morphology was visible. In *G. superba*, the extreme tip consisted of a more or less flat surface of cells, each around 60 μm in

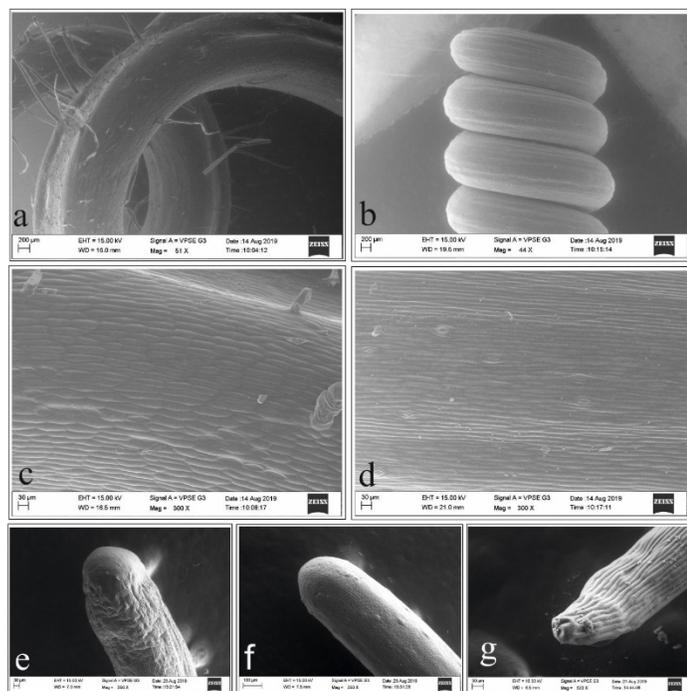diameter. Here, a spectacular cellular morphology similar to a "bundle of sticks" was revealed (Figure 2g).

Figure 2: SEM analysis of tendrils (a): *B. hispida* showing coils with trichomes (b): Closely appressed coils of *P. edulis* (c): epidermal features of *B. hispida* (d): Epidermal features of *P. edulis*. Note the presence of several stomata. Tendrils tips (e) *B. hispida* (f): *P. edulis* and (g): *G. superba*

Light microscopic and SEM analysis of the longitudinal sections of tendrils revealed that anatomically there exists a hierarchical arrangement of various tissues.[9,10] The cellular arrangement consisted of (i) An outer single-layered epidermal cells (ii) a Sclerenchymatous hypodermal region (iii) a Collenchymatous region (iv) a Parenchymatous region (v) a Vascular region (vi) Cellulose microfibrils and (vii) Shock-absorbing air cavities (Figure 3 and 4). The outer epidermal cells were single-layered and consisted of an outer hydrophobic covering which displayed autofluorescence (Figure 3a, 3b, 3c). Sclerenchymatous region was prominent and multi-layered in *B. hispida* (Figure 2a, 3d). In *P. edulis* multilayered collenchymatous region was prominent (Figure 3f). The fourth hierarchical tissue arrangement consisted of spectacular parenchymatous cells with bulky air filling intercellular spaces (Figure 2a, 4d, e, f). Vascular regions comprised xylem and phloem elements (Figure 3c, 4d, 4e, 4f). In *B. hispida,* the tendril consisted of a central air cavity of diameter 100 µm (Figure 4d) which started from a few microns below the terminal part and continued throughout the tendril. In *P. edulis,* the central air cavity was narrow (Figure 4e). *P. edulis* longitudinal anatomy revealed large parenchymatous regions (Figure 3h). Cellulose fibrils were observed, which are arranged in the form of a helical shape which ran longitudinally throughout the tendril. In *B. hispida* it was a double-helical shape (Figure 3e,f). Ladder-shaped cellulose

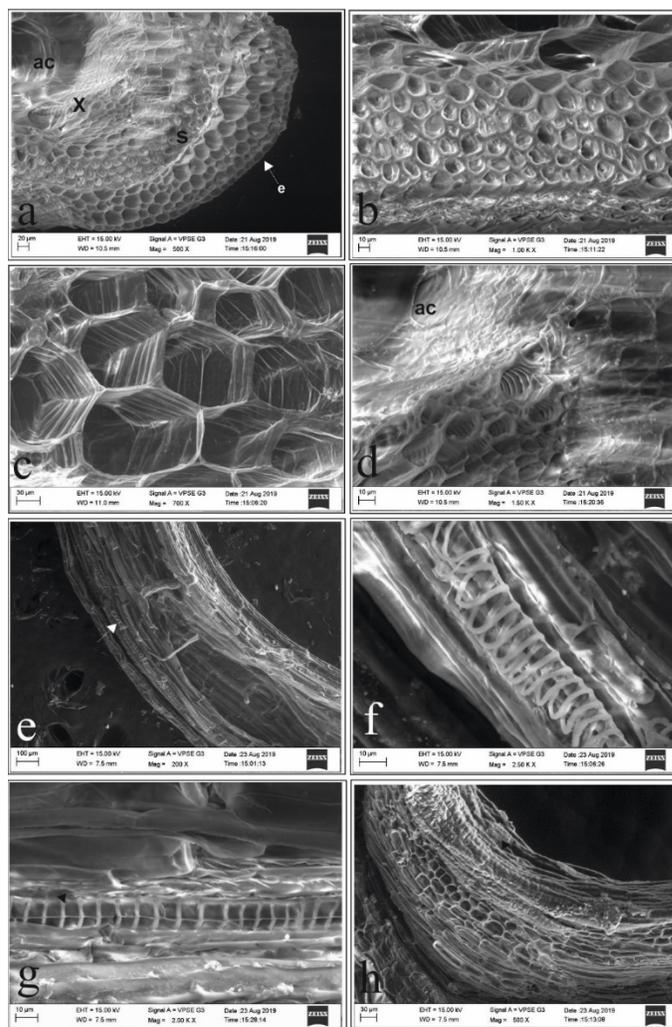microfibrils were also observed in the longitudinal section of *B. hispida* (Figure 3g).

Figure 3: Hierarchical tissue organizations *B. hispida* (a): Section showing the unilayer epidermis (e), sclerenchyma (s), xylem vessels (x) and air cavity (ac) (b): Collenchyma, the living mechanical tissue (c): Thickenings of xylem vessels (d): thickenings of xylem vessels and air cavity (e): Cellulose microfibrils, the structural framework of tendril in longitudinal sections (indicated by arrow) (f): Double helical arrangement of cellulose microfibrils (g): Another pattern of arrangement of cellulose microfibrils (h): Cellulose fibrils in *P. edulis*

G. superba tendrils were quandrangular in cross section and exhibited very large parenchyma cells. There was also air cavity towards the middle (Figure 4e). *P. edulis* also showed similar cellular organisation, but the fibrils were found to be covered with an unknown material so that the exact arrangement was unclear (Figure 3h, 4f). Being a leaf tendril, the cross-sectional shape was four-sided in *G. superba*. This tendril also possessed single-layered epidermis (Figure 4c), fluorescent outer hydrophobic deposition collenchyma at the corners, parenchyma and vascular tissues.[11]

AFM analysis showed that are characterised by a regular pattern of broad expanses on the cell surfaces, with dips in elevation between the cells (Figure 5). The average height of the bumps on

the surface (Ra) is 0.355nm. The Rq value noted is 0.48nm. The Rmax, the most significant single roughness depth obtained, was 5.09 nm.

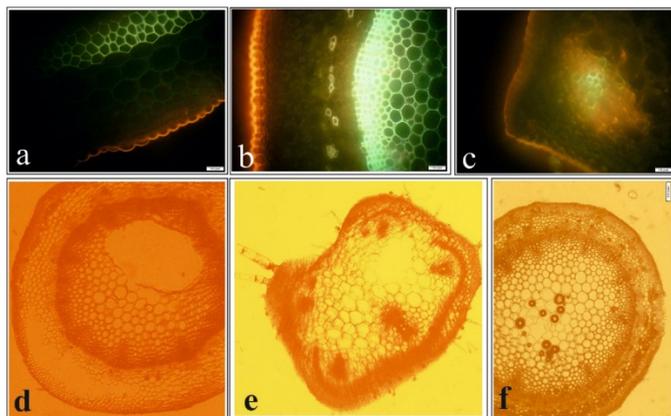**Figure 4**: Light and fluorescence microscopy of tendril structures. Autofluorescence in (a): *B. hispida* (b): *P. edulis,* and (c): *G. superba.* Note the specific Autofluorescence of the outer epidermal layer along the exterior and anticlinal walls. Cross sections of tendril showing the general architecture (d): *B hispida* with large central air cavity (e): Leaf tendril of *G. superba* and (f): *P. edulis* with small central air cavity.

Tendril is a typical example of one of the high-performance biological materials evolved for mechanical support and climbing adaptation in weak-stemmed plants.[5] Tendrils have been persistently an exciting subject of various analysts in different sciences such as mechanics, material science and designing zones since Darwin's time and much prior.

The fundamental mechanism of tendril coiling has remained elusive despite the long history of studying. It has been reported by a few authors that first the tendril finds some support and then starts to curl and finally forms the 'spring'. The stem or support cannot twist. The tendril coil formation occurs in a peculiar way that at some stage the spiral coils are inverted so that the tendril passes from the left to the right and then from right to left, and then the two turns divided by a tiny section known as "perversion".[12] As per the current study, the author has noted curling even without touching and sensing the support, as evidenced by many loose free coils.[13] According to Cogdell (2019),[14] similar free-coiling tendrils will also develop perversions.
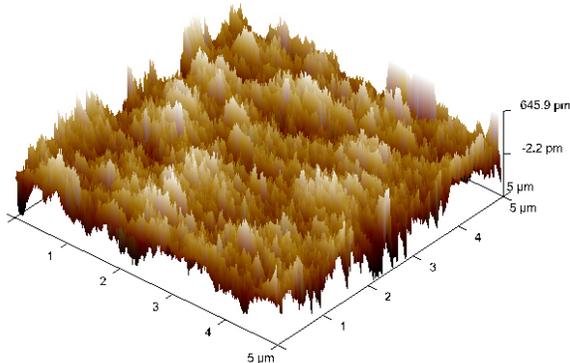

Figure 5: Results of the nanoscale surface design analysis of tendril in *B. hispida* by using AFM- 3D map.

In botanical aspects, tendril is just a morphological modification for support for climbing. However, when we consider the engineering aspects, the tendril is a multitasking organelle as evidenced by the occurrence of trichomes, stomata and chlorophyll apart from its primary function of support. The spring constant varied in the three samples selected. This indicated that the plant tendril efficiency is species-specific and is related to their respective habitats. *B. hispida* bears large fruits and hence may have to hold a more massive weight. Further, it is a broad-leaved plant. Hence wind pressure is higher. Further, it is weak-stemmed. These facts lead to the requirement of a more efficient tendril with a low spring constant.

The efficiency of the tendril is not just because of the coil or spring shape. Instead, it is contributed by a multitude of factors, especially the hierarchical arrangement levels evidenced in the anatomy of tendrils.[15] The outer coating provides hydrophobicity and protects the tendril from changing rigidity and elasticity by moisture absorption. Further, the occurrence of dead tissue sclerenchyma and the presence of living mechanical tissue collenchyma provides excellent strength and tension-bearing capacity. The sclerenchyma-collenchyma combination provides an additive mechanical strength. According to Brush (1912)[16], the collenchyma and sclerenchyma formation in mechanically disturbed seedlings is more than that of what is witnessed in undisturbed seedlings. This further confirms the tolerance of tendrils to mechanical stress and strain as it entails mechanical support rendering cell combinations. Another fact seen in the current study is the occurrence of spindle-shaped cells forming the epidermal outer covering in tendrils. It is interesting to note that muscle cells are also spindle-shaped in animals which are also tension-bearing and are capable of expansion and contraction.[17]

The structural component of the tendril may be provided mainly through the cellulose double helices. According to Wang *et al*. (2013)[18], similar helical structures enable the coil to bear tension on stretching and expanding in Tower Gourd plants. The study also concludes that there exist six-length scales of hierarchical chirality[18] ranging from basic sugar unit, cellulose molecule, cellulose microfibrils, the cellulose helix, the cell bundle and the tendril helix.[19] The current study also provides evidence to support this theory. Apart from this, the current study also proposes the role of air pockets in absorbing the tension. This manuscript is the first report which uses AFM in understanding the roughness and surface texture of a tendril. The nanosurface texture is essential to scrutinise as it serves us to corroborate the surface area and thereby predict the friction and the heaviness-bearing capacity of tendrils.[20]

CONCLUSIONS

Tendrils in plants are unique, specialised organs which display diverse morpho-anatomical properties. The architectural organization has specialised features exclusively serving the weak stemmed plants to photosynthesize better and propagate well. The cellular organisation of tendrils at the nanoscale level also would give us clues to redesign and upgrade machines with sensory perception notably in robotics. To achieve this, just mimicking the shape will not suffice, further it also requires an extraordinary design with different hierarchical arrangements. AFM can be

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employed to envisage the nanoscale surface design of tendrils. In summary, the hierarchical arrangement levels observed in the anatomy of tendrils, from their macroscopic coiling patterns down to the molecular structure, provide a compelling source of inspiration for robotics. By closely examining and emulating these natural systems, we can unlock new possibilities in robotics development, leading to more efficient, adaptable, and robust machines. The tension-bearing capacity demonstrated by tendrils, a fundamental trait derived from their hierarchical structure, holds the potential to revolutionise the way we design and construct robotic components. The combination of living mechanical tissues viz., collenchyma and dead tissue sclerenchyma, results in exceptional strength and tension-bearing ability. These living structures exhibit a seamless fusion of strength and flexibility, qualities that are often challenging to achieve simultaneously in artificial systems. By studying and integrating these principles, we can create robots that are not only capable of bearing significant loads but can also adapt to variable conditions with ease. As we delve deeper into the intricacies of tendril anatomy and function, we uncover knowledge that can inform the development of robotic appendages, grippers, and other components. This bioinspired approach can potentially enhance the capabilities of robots across various domains, from manufacturing and construction to space exploration and healthcare.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

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