

## A first report on pulvinar wrist-joint and foliar nyctinasty in *Malachra capitata* (L). L. (Malvaceae)

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### Abstract

Leaf closing and opening movements were observed in *Malachra capitata* (Malvaceae) that followed a diurnal circadian rhythm in Sundarbans, West Bengal. However, the presence of pulvinus that is a prerequisite for such movement was not conspicuous morphologically on the dorsal surface of the leaf petiole. A paired T test at  $p=0.05$  evinced significant difference in the collar diameter of the petiole base and its distal end on the ventral surface. The anatomical study of the petiole exhibited a eustele or dissected siphonostele. The collateral vascular bundles of the petiole were arranged in a single ring that was interrupted at several places with leaf gaps. On the other hand, the transverse section of the distal end of the petiole that appeared as an asymmetric swollen mass at the base of the leaf lamina exhibited an ectophloic siphonostele. The ectophloic siphonostele had a concentric ring of outer phloem facing the epidermis, inner xylem facing the central pith like collenchymatous parenchyma and a broad parenchymatous cortex surrounding the entire solid stele. This distal-end of the petiole or pulvinus facilitated a wrist-joint movement for leaf closing or upward vertical movement in the evening and leaf opening or horizontal movement in the morning. This is the first report of pulvinus at the distal end of the petiole that mediates night sleep or foliar nyctinasty in *M. capitata*.

**Keywords:** ectophloic, eustele, distal, petiole, pulvinus, siphonostele, solid stele.

### Introduction

*Malachra capitata* (L). L. of the family Malvaceae is also called Brazil jute and is a native of Tropical America. It grows commonly in wastelands, abandoned fields and on road sides in many places in India. The fibre of the plant is of economic importance (Sivarajan & Pradeep, 1996). It has several medicinal properties to treat

pain, hepatic cirrhosis, inflammation, diarrhoea, cutaneous disease, and others (Deodhar, 2016; Hooker, 1874).

The leaves of a natural population of *M. capitata* were noticed to exhibit leaf closing movements at sunset in a field of Sundarbans, West Bengal, India. The same leaves returned back to their original position at sunrise. Such circadian rhythmic opening and closing of the leaf

is called nyctinasty (Darwin, 1880). Species exhibiting nyctinasty in many angiospermic families show a common character at the base of the petiole called the pulvinus. However, the presence of pulvinus was not described in the taxonomic description of *M. capitata* nor was it listed in the list of plants exhibiting nyctinasty. Hence, the present study is attempted to investigate the presence of pulvinus in the petiole of *M. capitata* and its implication on nyctinastic behaviour of the species.

### Materials and Methods

The study area was situated in Gobardhanpur, Dhonchi (21.61081° N, 88.39565° E) of 24 South Parganas, Sundarbans, West Bengal, India. The village is close to Bay of Bengal and has better infrastructural facilities in terms of roads, building and schools compared to nearby villages. *M. capitata* population were found growing abundantly along road sides and fallow land. A natural plant population of *M. capitata* growing by the roadside was selected for ascertaining the presence of pulvinus in the leaf petiole of the species.

Twenty plants were tagged for identifying the pulvinus in the leaf petiole of the species. These plants were observed at 6 hours interval for 25 days to monitor its night sleep. Morphologically the distal end of the petiole at the base of the leaf lamina appeared different. A vernier calliper was used to measure the length and the collar diameter at the base of the petiole and the probable pulvinar region at its distal end. Standard double staining of the transverse section of petiole and its distal end were carried out by collecting leaf samples randomly. The samples were fixed in 3:1 acetyl aldehyde solution (70%

alcohol: 30% acetic acid). The sections from the base and distal end of the petiole were stained with safranin and alcian blue, and passed through different grades of ethanol for dehydration. Good sections were mounted on slides with euparal and observed under microscope for anatomical investigation. Digital photographs were taken of the sections for further study. Literature survey was carried out to ascertain any mention of the pulvinus in the holotype of the species. Other taxonomic descriptions of the species in various Floras and research papers were examined. Specimen of the plant species was photographed, collected and pressed for future reference.

### Results and Discussion

In *M. capitata* the distal end of the petiole appeared as an asymmetric swollen mass of tissue or a bump on its lower surface (Fig.1A & B). This structure was similar to the pulvinus that acts as a motor at the base of the leaf lamina facilitating leaf closing and opening also called foliar nyctinasty (night sleep). However, in most nyctinastic species like that of Leguminosae the pulvinus is situated at the base of the petiole (Darwin, 1880). In *M. capitata*, it was difficult to morphologically distinguish the pulvinus from the petiole, especially in younger leaves. Hence it was debatable whether the swollen tissue at the distal end of the petiole was pulvinar or non-pulvinar in nature?

*M. capitata* (L.) L. was first validly published by Linnaeus (1767). The accepted name of the species comes from its basionym *Sida capitata* also given by Linnaeus (1753). There are nine synonyms of the species given by various authors. The description given by Linnaeus (1767)



**Fig. 1.** Petiole and pulvinus of leaf (A & B) and leaf closing (C) in *M. capitata*; Longitudinal section of the asymmetric pulvinus in North-South median showing the bulge in the Vascular tissue (D) and without bump in the East-West median (E)

describes the plant as a herb or woody shrub with 'triphylis' or 3 lobed leaf. There was no mention of a pulvinus in the species. Various other authors have also described the leaf of *M. capitata* and its associated features over the years (Prain, 1800; Wiggins & Porte, 1971; Basu & Bose, 1971; Stewart, 1972; Heagy, 1993; Fryxell & Hill, 1993; Sivarajan & Pradeep, 1996; Issac Kehimkar, 2001; Pullaiah, 2006; Sasidharan, 2015). However, none of the descriptions have any mention of the pulvinus in the leaf petiole of the species.

A detailed morphological and anatomical observation was carried out to ascertain the distal or pulvinar region of the *M. capitata* leaf petiole. Measurements recorded the average petiole length as  $6.6 \pm 1.9$  cm, whereas the distal end of the petiole had an average length of  $0.6 \pm 0.1$  cm. Only, about 10% of the total petiole length was occupied by the distal end. The petiole of *M. capitata* is not a complete cylinder. On the dorsal surface, the petiole has a straight shallow furrow from its base to its distal end making it slightly depressed. On the ventral surface, the entire length of the petiole is moderately rounded except at its distal end where the petiole tissue becomes highly rounded and appears swollen. This results in an asymmetry in the transverse plane of the petiole. Two different sets of measurements were recorded of the collar diameter for both the petiole base and its distal end depending on their plane of measurement (Table 1). In horizontal position, the measurement of the petiole from right to left or East-West (EW) plane; with the dorsal leaf lamina facing upward; evinces average collar diameter of the petiole base as  $0.42 \pm 0.09$  cm and that of its distal end as  $0.37 \pm 0.07$  cm. The same petiole in the same position when measured from above to below or North-

South (NS) plane, recorded the average collar diameter of petiole base as  $0.35 \pm 0.08$  cm and that of its distal end as  $0.43 \pm 0.08$  cm. A paired T-test at 5% probability expressed the difference between the means of the collar diameter of the petiole base and its distal end to be statistically insignificant when measured in E-W plane. However, the same was not true for the collar diameter of petiole base and its corresponding distal end when measured in the N-S plane (Table 1). The size of the distal end of the petiole in the NS plane was significantly bigger than its base. This result brings forth the importance of the plane of measurement of the collar diameter between the petiole base and its corresponding distal end. Hence, morphologically the dorsal surface of the petiole base and its distal end may not be conspicuously different. But, the ventral surface of the petiole exhibits a significant distinction between its two ends, if observed closely (Fig. 1B). It was also imperative to analyse whether there was any significant difference in the collar diameter of the same petiole base when measured in two different planes (EW-NS). The same question was applicable for the collar diameter of the distal end of the petiole. A paired T-test at  $p=0.05$  evinced that the collar diameter of the petiole base of the species would be significantly different when measured in the EW plane as compared to that measured in NS plane. The same was true for the collar diameter of its distal end when measured in two different planes (Table 1). The longitudinal section of the distal end of the petiole shows a significant bulge in the vascular tissue when examined in the NS plane as compared to the ES plane (Fig. 1D & 1E).

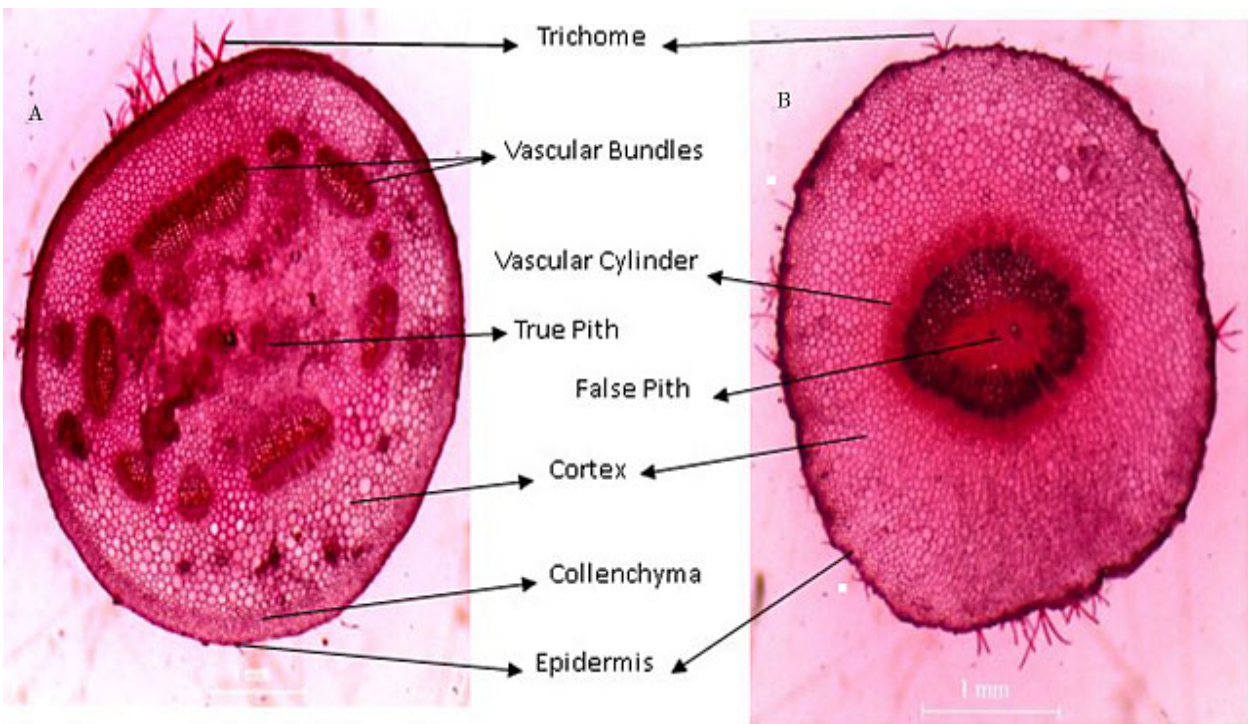
The anatomical investigation of the petiole base and the proposed distal pulvinar region (henceforth referred as

pulvinus) evinced a distinct difference in the layout of their tissue system. A transverse section of the basal region of the petiole evinces a eustele or dissected siphonostele with large collateral vascular bundles intercepted by leaf gaps and accessory smaller vascular bundles arranged in a single ring (Fig. 2A). The smaller bundles are called meristele. Each vascular bundle has an outer phloem with phloem fibre and inner xylem with protoxylem and metaxylem (Fig. 3A). In the petiole base, the epidermal region was followed by a collenchyma of 4 to 5 layer of cells, broad parenchymatous cortex and a parenchymatous pith (true pith) in the

petiole. The petiolar vascular bundles can be of many types in a genus but are specific to a species (Nurul-Aini et al., 2013). On the other hand, the transverse section of the pulvinus shows an ectophloic siphonostele with central concentric vascular bundle forming a solid stele surrounded by a broad parenchymatous cortex. The stele has an outer phloem ring facing the epidermis and an inner xylem ring facing the central solid pith-like region. The pith like region was composed of collenchymatous parenchyma cells and can be considered as a false pith (Fig 2B). The elastic nature of the collenchyma tissues within the vascular cylinder may

**Table 1.** Statistical analysis of collar diameter (cm) of petiole base and its distal end in *M. capitata*

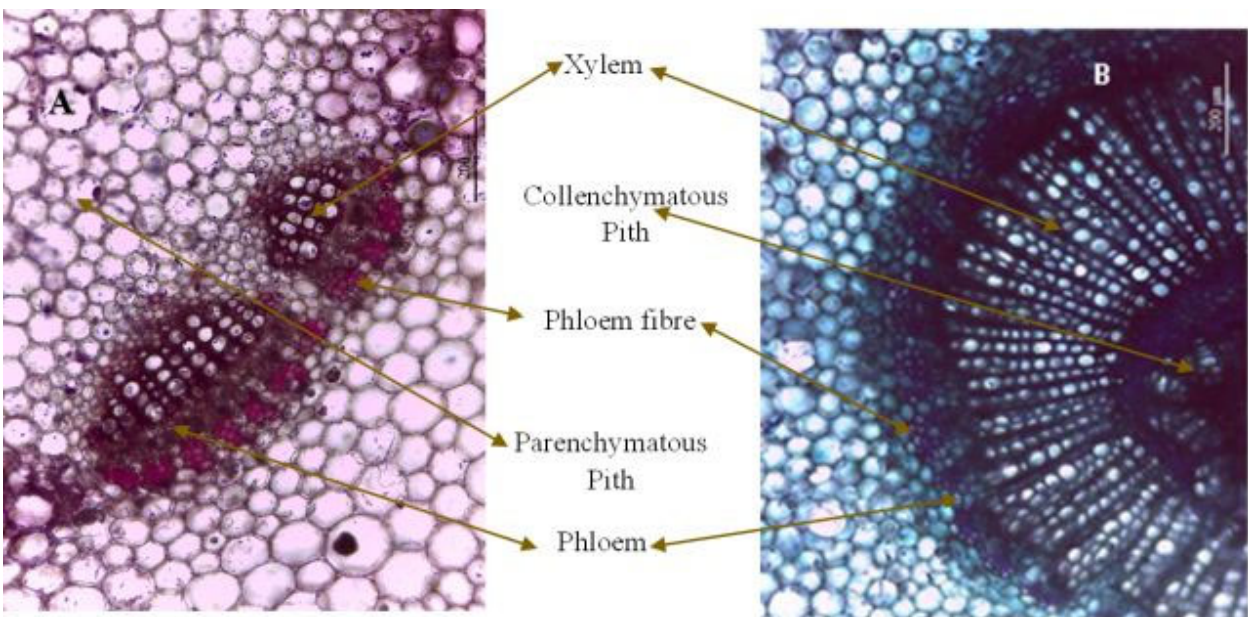
Collar diameter (cm)	Petiole base	Petiole distal end (Pulvinus)	Petiole base	Petiole distal end (Pulvinus)
<b>Measurement plane</b>	<b>East-West (Right to Left)</b>		<b>North-South (Above to Below)</b>	
<b>N</b>	20	20	20	20
<b>Mean</b>	0.4200 (x)	0.3725 (y)	0.3465 (x <sup>1</sup> )	0.4295(y <sup>1</sup> )
<b>SD</b>	0.0951	0.0749	0.0809	0.0821
<b>SEM</b>	0.0213	0.0167	0.0181	0.0184
<b>Null hypothesis</b>	<b>x-y=0</b>		<b>x<sup>1</sup>-y<sup>1</sup>=0</b>	
<b>Paired T-test</b>	Significance level	Difference in mean	Significance level	Difference in mean
<b>t</b>	2.03	0.18	-2.02	-3.22
<b>Probability</b>	5%	8.78% (≠5%)	5%	0.26% (<5%)
<b>Inference</b>	<b>Null accepted</b> <b>x-y=0</b>		<b>Null rejected</b> <b>x<sup>1</sup>- y<sup>1</sup>=0</b>	
Collar diameter (cm)	Petiole base		Petiole distal end (Pulvinus)	
<b>Plane of Measurement</b>	<b>EW</b>	<b>NS</b>	<b>EW</b>	<b>NS</b>
<b>Mean</b>	0.4200 (x)	0.3465 (x <sup>1</sup> )	0.3725 (y)	0.4295(y <sup>1</sup> )
<b>Null hypothesis</b>	<b>x- x<sup>1</sup>=0</b>		<b>y-y<sup>1</sup>=0</b>	
<b>Paired T-test</b>	Significance level	Difference in mean	Significance level	Difference in mean
<b>t</b>	-2.03	-2.63	2.02	2.29
<b>Probability</b>	5%	1.23% (<5%)	5%	2.75% (<5%)
<b>Inference</b>	<b>Null rejected</b> <b>x- x<sup>1</sup>=0</b>		<b>Null rejected</b> <b>y-y<sup>1</sup>=0</b>	



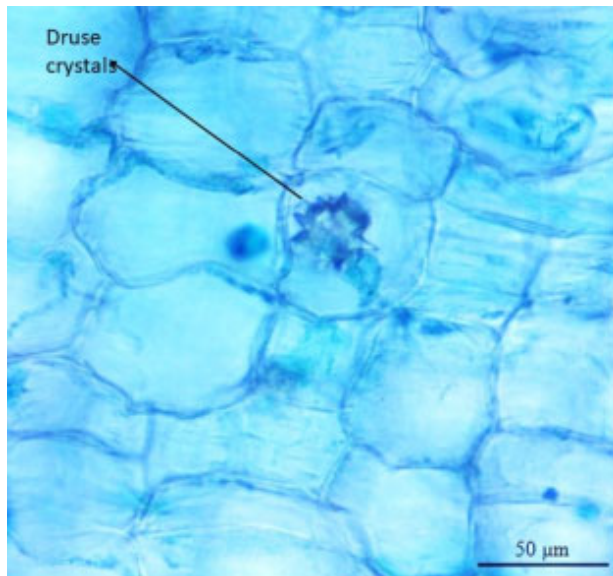
**Fig. 2.** Transverse section of petiole (A) and pulvinus (B) of *M. capitata*

offer additional tensile strength and flexibility in movement of the pulvinus. The phloem of the pulvinus has prominent phloem fibre as in the petiole (Fig. 3B).

The prominent phloem fibre tissue in the petiole and pulvinus would provide mechanical strength to the vascular bundles. The phloem fibre tissue or bast



**Fig. 3.** Vascular bundle in petiole (A) and pulvinus (B) of *M. capitata*

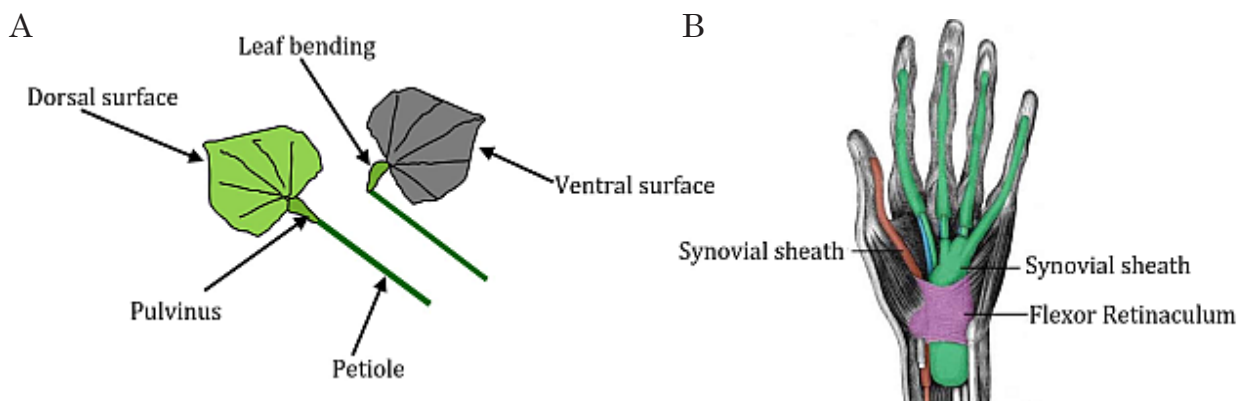


**Fig. 4.** Stellated dodecahedron druse crystals in parenchyma cells of petiole and pulvinus of *M. capitata*

in the vascular bundle of the species is what makes it a good source of fibre like the Indian jute (*Corchorus olitorius*). The presence of stellated dodecahedron druse crystals was observed in the ground tissue of both the petiole and the pulvinus of *M. capitata* (Fig. 4). Likewise, the presence of trichomes was observed on the epidermis of the entire petiole and

pulvinus. Similar anatomical traits have been observed in the pulvinus of several nyctinastic plants (Fuhrman & Koukkari, 1981; Rodrigues & Machado, 2008). The anatomical differences in the vascular bundle of the base of the petiole and its distal end confirm the distinct functional and structural differentiation of the petiole and the pulvinus in *M. capitata*.

The functional importance of the presence of pulvinus at the distal end of the petiole lies in its ability to act as a hinge between the petiole and the leaf lamina to facilitate leaf movement. The observation of the tagged plants at 6 hours interval evinced that the leaves of *M. capitata* opened at sunrise (horizontal position) and closed (vertically upward position) at sunset (Fig. 1C). The presence of the pulvinus and its positioning at the apex of the petiole allows a wrist-joint like movement to enable leaf closing and opening independent of the petiole (Fig 5 A). This phenomenon is similar to the function of the flexor retinaculum of the human wrist (Fig 5 B) that efficiently controls the up-down movement of the entire palm and fingers without moving the lower arm (Sapin et al., 2019). In most



**Fig. 5.** Leaf bending by the pulvinus of *M. capitata* at sunset (A), wrist bending by the flexor retinaculum in human wrist (B)

nyctinastic species where the pulvinus is present at the proximal end or base of the leaf petiole the movement is controlled by an elbow joint like movement involving petiole, pulvinus and leaf blade. The leaf closing phenomenon is controlled by osmotic motor of the pulvinar tissue. Mayer & Hampp (1995) reported that the parenchymatous cells surrounding the vascular bundle in the pulvinus act as motor cells that have two functionally different tissues, the adaxial extensor and the abaxial flexor. Thus, the oscillations in leaf movement of nyctinastic species is generated by the rhythmic osmotic and plasmolytic conditions of motor cells of the pulvinus similar to the osmoregulation of the guard cells (Udea & Nakamura, 2007; Uehlein & Kaldenhoff, 2008). Hence, the closing of the leaf at sunset as observed in *M. capitata* is night sleep or foliar nyctinasty facilitated by the pulvinus located at the distal end of its petiole.

## Conclusion

This study establishes the presence of pulvinus at the distal end of the petiole in *M. capitata*. The pulvinus is a morphologically and anatomically distinct character of the petiole. The position of the pulvinus at the distal end of the petiole is unique in the species as it works like a wrist-joint. In most nyctinastic species the pulvinus is at the proximal end of the petiole providing elbow-joint leaf movement. Such wrist jointed pulvinus could be an advantageous ecological adaptation of the species. The reference of the pulvinus in future taxonomic descriptions may be stated as 'the pulvinus is a swollen tissue on the distal end of the petiole in *M. capitata*. The pulvinus facilitates the upward and downward

movement of the leaf in response to sunlight exhibiting foliar nyctinasty in the species. The mention of the pulvinus in the taxonomic description of *M. capitata* can be a useful character for the identification of the species. It is also important to mention the pulvinus in the description of the species to highlight its physiological and ecological significance as a nyctinastic species. *M. capitata* is an exotic species and its presence in the vegetation of Sundarbans poses a risk to the mangrove ecosystem. The modification of the pulvinar functions of the species may help in reducing its invasive attribute. Hence, this study provides new areas of research on the morphological, anatomical, taxonomical, physiological and ecological importance of pulvinus in *M. capitata*.

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