



Research Article

Foliar micromorphological character studies on *Trichosanthes* L. (Cucurbitaceae) from Terai & Duars, West Bengal, India.

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Abstract: *Trichosanthes* is one of the most economically important genera of the family Cucurbitaceae. Studies on trichome micro-morphology and stomata were investigated for six species of *Trichosanthes*. All species has been recorded from the Terai and Duars of West Bengal. Range of variability in *Trichosanthes* species are quite variable, trichomes types including uni-multiseriate, macro-microform, conical, candelabra, capitated, glandular and non-glandular. Stomatal character shows variability (Paracytic, Actinocytic, Anomocytic and Diacytic). Trichomes and stomatal characters are utilised for phenetic studies which found to be important taxonomic tools to solve any disputes at least at or below species level.

Keywords: Stomata; Taxonomic significance; *Trichosanthes*; Trichomes; Cluster analysis

Introduction

Lamina being important part of plant acquire a good number of anatomical and micro-morphological features that are most varied in angiosperms. Though these can be employed as needful taxonomic delimitations (Metcalf 1968). Foliar micro-morphological characters, especially the trichomes and stomata are considered as some of the primary diagnostic features in segregating the major groups of plants and epidermal characters are used successfully in the delimitation of a number of taxa (Chauhan and Daniel, 2011). Different types of glandular and non-glandular trichomes are important diagnostic features of members of Cucurbitaceae and around more than 300 descriptions of trichome types are recorded in different botanical literatures (Kolb and Müller, 2004). The elaborate study on structure and distribution pattern of trichomes among the different genus of Cucurbitaceae have received considerable attention of various earlier workers (Inamdar *et al.*, 1990, Ibrahim 2003, Kolb and Muller 2004, Inamdar and Gangadhara 2008, Ali and Fahad 2011, Abdulrahman *et al.*, 2011, Adebooye *et al.*, 2012, Bibi and Okoli 2014). *Trichosanthes* Linnaeus, (Sp. Pl. 2:1008.1753) is the most interesting and economically valuable genera of Cucurbitaceae. The fruits, flowers and tender shoots of *Trichosanthes dioica* and *Trichosanthes cucumerina* are quite popular vegetables in India especially in North East India. For this genus 206 scientific names are available, of which 37 are accepted species along with 14 scientific plant names of infraspecific rank (theplantlist.org). Mitra *et al.*, (2005), reported around 22 species of *Trichosanthes* from territory of India and out of that

10 species from West Bengal. 21 species are mostly concentrated to the Malabar Coast in Western-Ghats and North-Eastern region of India (Rai *et al.*, 2008). Study of overall foliar micro-morphological character of this genus is variable among the species level. The trichomes and stomatal characters and their comparative analysis for the genus *Trichosanthes* is less explored. Hence, the present communication deals with micro-morphology of six species of *Trichosanthes* of Cucurbitaceae, quite common in wild and cultivated in Terai and Duars parts of West Bengal, India.

Materials and Methods

To study the variation of foliar epidermal micro morphology of six species of *Trichosanthes* [*T. lepiniana* Mohitnagar, 012, dated 12.05.2016; *T. tricuspidata* Sukna, 021, dated 25.05.2016; *T. quinqueangulata* Haldibari, 019, dated 20.05.2016; *T. dioica* Mandalghat, 013, dated 12.05.2016; *T. cucumerina* Dhabganj, 020, dated 20.05.2016; *T. mallichiana* Kurseong, 025, dated 25.05.2016] microscopic anatomical observations were carried out following the standard method (Metcalf, 1950; Esau, 1959 and Creedy, 1977). Fresh and fertile specimens of *Trichosanthes* sp. were collected from different habitats of the study area. About 2.5 cm long foliar parts were taken peeled from both epidermal surfaces and then mounted. Camera Lucida drawings were done and measurements of the stomata, guard cells and trichomes were recorded under standardized ocular micrometre. The photographs were taken under the Magnus microscope and Leica DM LS 2 microscope with 10 X & 40 X objectives. For studying trichomes

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epidermal peels were taken out manually using blade and forceps by scrapping method and for stomatal studies maceration of cuticles in conc. nitric acid were done. Finally, the specimens were rinsed in distilled water, stained with 1% aqueous safranin solution and mounted in 5% glycerine. Different Stomatal parameters including stomatal type, stomatal frequency, stomatal index and stomatal size (Dilcher, 1974) and frequency of each complex type were expressed as percentage occurrence of such complex type based on all occurrences (Obiremi & Oladele, 2001). Epidermal cell size was determined as product of length and breadth (Philips, 1959). All the voucher specimens were deposited at NBU Herbarium. For Hierarchical cluster analysis XLSTAT 2014.5.03 software is used. Similarity is weighed based on Pearson's Correlation Coefficient.

Results

Foliar Micro-morphological evidences in the six species of the genus *Trichosanthes* seems to be

enough diagnostic values for delimiting species studied. The foliar micro-morphological features were analysed by its qualitative and quantitative characters. Detailed variable shapes, size of trichomes and stomata for all the collected species of *Trichosanthes* examined carefully. Four types of stomata's were recorded Anomocytic (*T. quinquangulata*, *T. dioica*, male), Actinocytic (*T. tricuspidata*), Paracytic (*T. lepiniana* & *T. dioica*, female) and Diacytic (*T. cucumerina* & *T. wallichiana*). In *T. lepiniana*, *T. cucumerina* and *T. wallichiana*, *T. dioica* (male) stomata present only in abaxial surface. Whereas, in *T. tricuspidata*, *T. quinquangulata*, *T. dioica* (Female) stomata are present on both surfaces. Quantitative Stomatal characters are important tool for determining taxonomic significance. Therefore, largest stomata are found in *T. dioica* female 13 O.D whereas smallest in *T. cucumerina* 5 O.D. The range of stomatal index was varying from 26.6 % in *Trichosanthes quinquangulata* to 9.52 % in *Trichosanthes cucumerina*. All the specimens comprise of non-glandular hair except female *T. dioica*.

Table 1. Stomatal and trichome parameters of *Trichosanthes* sp. (Vernacular names B: Bengali, E: English, H: Hindi)

Taxa	Stomatal Frequency (µm)	Stomatal index (%)	Stomatal type	Trichome type	No. of cells	Length (O.D)
<i>Trichosanthes lepiniana</i> (Naudin) Cogn. (E: Snake gourd)	526.31	18.75	Paracytic	Branched uniseriate marcoform candelabra hair	5 to many	20
<i>Trichosanthes tricuspidata</i> Loureiro (H: Mahakal, Indrayan)	349.3	18.6	Actinocytic	Unbranched, uniseriate macroform conical bend hair	4 – 5	80
<i>Trichosanthes quinquangulata</i> A. Gray	701.75	26.6	Anomocytic	Unbranched, uniseriate macroform conical and filiform capitated hair	4 – 7	30 – 70
<i>Trichosanthes dioica</i> Roxb. (E: Pointed gourd; B: Potol)	205.4	10.71	Anomocytic	Uniseriate macroform conical	2 – 3	30 – 95
<i>Trichosanthes cucumerina</i> L. (B: Chichinga)	273.9	16	Paracytic	Glandular hair	4 – 10	20 – 32
<i>Trichosanthes wallichiana</i> (Ser.) Wt.	350.89	9.52	Diacytic	Unbranched, uniseriate macroform conical hair	3	40
	174.6	17.27	Diacytic	Unbranched, uniseriate macroform conical hair	3	30 – 52

T. lepiniana trichomes have flattened bases and are distributed poorly over the adaxial surface. [PI-1: I-K]. In *T. cucumerina*, trichomes are densely distributed, thin walled, irregular in shape, without flattened disk at base, slightly blunt apex. [PI-1: A-D]. The trichomes of *T. wallichiana* are non-glandular, apex acute. [PI- 2: V-W]. *T. tricuspidata*, the trichomes are 2–3 celled with well-developed flattened disk at base, slightly curved at end with acute apex and are without cystolithic appendages. [PI-2: Q-U]. The trichomes in are glandular and non-glandular, acuminate and capitated in case of *T. quinquangulata* [PI-2: M-P]. Interestingly male macroform conical hairs of *T. dioica* are 2 – 3 celled, with prominent flattened disk of two rows of cells and female have 4-10 celled glandular hair [PI-1: E-H]. The six species can further be classified into two groups on the basis of trichome types namely (a) those in which trichomes branched (*T. lepiniana*) and (b) those in which trichomes unbranched (rest of five species).

Discussion

Trichome is the most significant taxonomic features for evaluating utility of the plant specimen as it is a major tool for plant's physical defence. In studied species of *Trichosanthes* a wide range of trichomes are found. The glandular trichomes are exclusive character of female plant in case of *Trichosanthes*. Glandular hairs developed as physical defence mechanism form. If foliar micromorphology is performed on *Trichosanthes dioica* without bloom by presence or absence of glandular hair one can easily drag conclusion on its identity. Presently, six trichome types are recorded respectively: uniseriate macroform conical, branched uniseriate marcoform candelabra hair, uniseriate macroform conical bend hair, uniseriate macroform filiform capitated hairs. uniseriate macroform conical and glandular trichomes. Trichomes are mostly present on adaxial surface in all the studied taxa. Exclusive trichome types like filiform capitated trichomes are observed

in *T. quinqueangulata*. In *T. tricuspidata*, trichomes are uniseriate macroform bend conical shaped. Branched, uniseriate marcoform candelabra trichomes are observed only in *Trichosanthes lepiniana*.

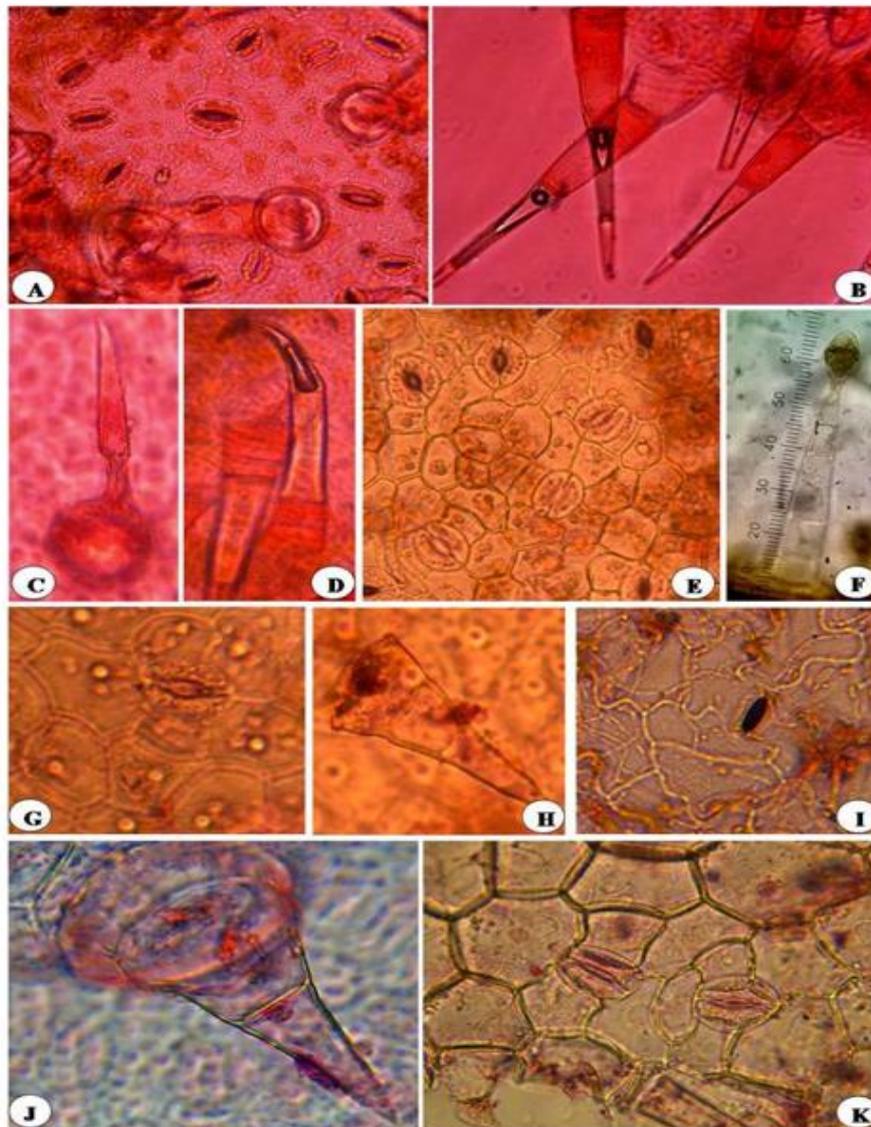


Plate 1. A & B Abaxial stomata & trichome C & D Adaxial trichome of *Trichosanthes cucumerina*; E & F Abaxial stomata & trichome G & H Adaxial stomata & trichome of *Trichosanthes dioica*; I & J Abaxial stomata & trichome K. Adaxial stomata of *Trichosanthes lepiniana*

Taxonomic significance of epidermal characters including epidermal cell architecture, such as the stomatal complex and surface ornamentation have been used in some cases as a substitute method to classify at the genus as well as species level (Sharma & Dunn 1969; Stace 1965). On the other hand, six species categorized on the basis of stomatal structure and distribution. Three species are amphistomatic except *T. lepiniana*, *T. mallichiana* and *T. cucumerina* where the stomata are restricted on abaxial surface. Amphistomatic stomata are actinocytic and anomocytic whereas abaxial surface stomata are diacytic and paracytic. This amphistomatic nature is one such character which can aid to taxonomic identification and systematic classification.

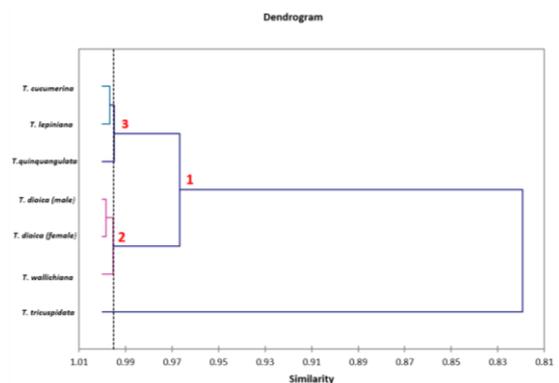


Figure 1. Dendrogram of six species of *Trichosanthes* (male and female data of *T. dioica* included separately)

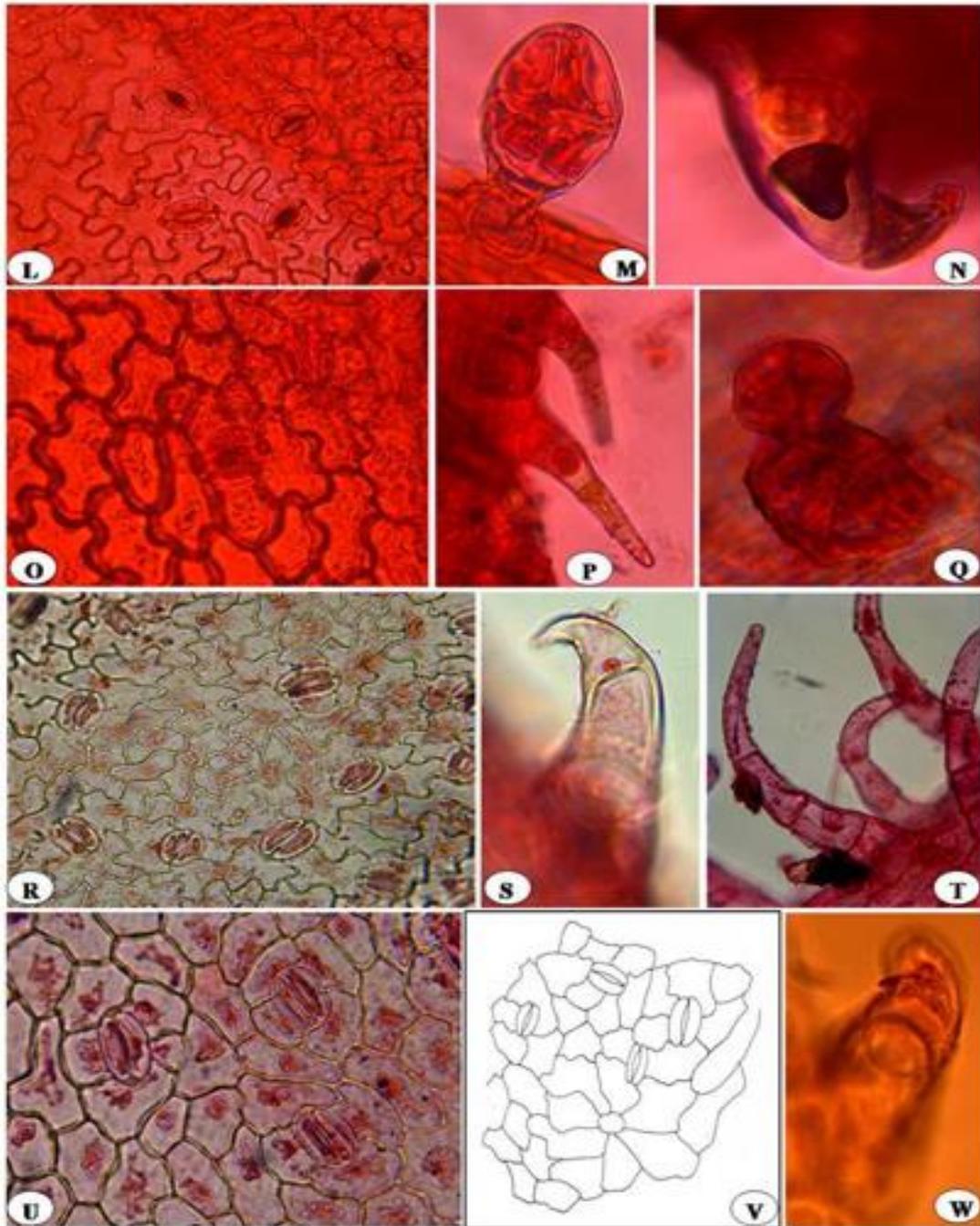


Plate 2. L. Abaxial stomata M & N Abaxial trichome O & P Adaxial stomata & trichome *T. quinquangulata*; Q & S Abaxial trichome R Abaxial stomata T & U Adaxial trichome & stomata *T. tricuspidata*; V. Camera lucida drawing of abaxial stomata W Abaxial trichome of *T. wallichiana*

The diversity of foliar micro-morphological features was based on the variation of trichome types and stomata types. All of these features are very conserve for specific species and can be helpful for authentic identification of *Trichosanthes* species. The hierarchical clustering (Fig.1) showed three constant clustered groups based on similarity of leaf micro-morphology including stomata and trichome. The very first clad of *T. tricuspidata* bifurcates and stands odd out of even because the clustering separates *T. tricuspidata* and the remaining *Trichosanthes* species on other branch (1) showing

the maximum dissimilarity and primitiveness from the rest. The branch 1 separates into two groups one with three species *T. dioica* (male and female) and *T. wallichiana* and other with three species *T. lepiniana*, *T. cucumerina* and *T. quinquangulata*. The three-species come off the branch that goes up from branch 1 to 2 showing more proximity. The branch 2 further splits into two lines one clad having *T. dioica* male and female showing highest similarity than other clad of *T. wallichiana*. It seems that the male and female species are developed simultaneously. The branch 3 splits into two clads

where *T.cucumerina* and *T.lepiniana* of first clad shares more similarity than *T.quinquangulata* of second clad.

Species key of six species of *Trichosanthes*

- 1a. Trichomes branched, stomata paracytic *T. lepiniana*
 1b. Trichomes unbranched, stomata non-paracytic 2
 2a. Trichomes with glandular hairs, less than 3 celled *T. dioica*
 2b. Trichomes without glandular hairs, more than 3 celled 3
 3a. Conical and filiform capitated hairs present, stomatal frequency more than 700 μm
*T.quinquangulata*
 3b. Only conical hairs present, stomatal frequency less than 700 μm 4
 4a. Stomata present on both surfaces, presence of conical bend hair*T. tricuspidata*
 4b. Stomata present on single surface, absence of conical bend hair 5
 5a. Stomatal index less than 10, trichome length less than 50 O.D *T. cucumerina*
 5b. Stomatal index more than 10, trichome length more than 50 O.D *T. wallichiana*

Conclusion

Based on foliar micro-morphological features, species of the genus *Trichosanthes* can readily be distinguished from one another. Such foliar micro-morphological features as on trichome and stomata are thus useful tools for diagnostic and other taxonomic works without blooms. This approach can be put to use in several disciplines of biological science and by this approach we can easily documented the micro-morphological data for further uses. The enumerated characteristics of trichomes and stomata have potential taxonomic capability for authentication of species and below rank identification and also for power drugs of medicinally important *Trichosanthes* and their adulterants. Not only that, phenetic studies provides great impact finder evidence on their species level affinity. The cluster analysis reveals the closest affinity within *T.cucumerina* and *T.lepiniana*; *T.dioica* male and *T.dioica* female. The separation of male and female clad hypothesised parallel evolution. Whereas, *T.tricuspidata* shows maximum dissimilarity proving evolved as one of that alone within the other species studied.

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