Original Research Article

Diversity of trichomes in Calliandra haematocephala Hassk. (Caesalpinioideae DC., Fabaceae Lindl.)

ABSTRACT

Objective: High structural diversity of trichomes in the members of family Fabaceae Lindl. has been a subject of study from decades. The present study was aimed to study structural diversity of trichomes in Power Puff Tree (*Calliandra haematocephala*, Caesalpinioideae, Fabaceae) a pan-tropical ornamental tree
Methods: Plant specimens were collected from the campus of Govind Ballabh Pant University of Agriculture and Technology Pantnagar (India) during the year 2020. Plant species was identified with the help of relevant Floras. Fresh plant material was used for vestiture and trichome study under light microscope using standard anatomical procedures. Illustrations of trichomes were drawn by using prism type camera lucida and photographs were taken at different magnifications. Standard terminology of trichomes (Payne, 1978) was adopted to describe different structural types.
Results: Nine different types of glandular and non-glandular trichomes were recorded in *Calliandra haematocephala* Hassk. Four different vestiture types were recorded on the surfaces of studied parts. Trichomes were found present in all vegetative parts and some reproductive parts *viz*; peduncle, bracts, bracteole, petals while other reproductive parts like sepals, filaments, anther, ovary, style and stigma were found completely glabrous.

Keywords: **Keywords: *Calliandra*; *Trichomes*; *Vestiture*; *Glandular*; *Non-glandular*.**

Comment [F1]: Name of floras

Comment [F2]: Trichomes were described according to standard terminology by Payne, 1978

Comment [F3]: The abstract did not clearly state the main issues though it provides a good introduction to the work

Comment [F4]: Keywords should be written in alphabetical order

1. INTRODUCTION

A well established term in botanical literature 'trichome' refers to a kind of epidermal outgrowth or appendages of a plant surface which are of diverse form, structure and function (1). Trichomes may occur on all parts of a plant, either they persist throughout the life of an organ, or they are ephemeral. Trichome types have been successfully used in the classification o genera and even of species in certain families and in the recognition of interspecific hybri (2,3,4). Trichomes are micromorphological characters which can be used to identify plant species when the plant lacks its floral structures (5,6). *Calliandra haematocephala* Hassk. is a

Comment [F5]: This sentence needs to be recast This first sentence drifted away from what the abstract focused on

member of genus *Calliandra* belonging to subfamily Caesalpinioideae DC. of the family Fabaceae. *Calliandra haematocephala* is a 1-3 meters high evergreen, shrub, with spreading and pendulous branches, forming a dense round head. Leaves are alternate, stipulate, petiolate, compound and bipinnate. Flowers are sessile and arranged in small, dense flower heads, watermelon pink with numerous silky stamens, fruit is a compressed legume (7) (Figure.1). Various pharmacological properties of leaves have been reported in *Calliandra haematocephala* such as analgesic, anticonvulsant, antipyretic, anti-ulcer and antioxidant (8, 9). Leaf extracts of *Calliandra haematocephala* have antiviral activity against RV infection *in-vitro* (9).Leaves of *Calliandra haematocephala* have been found to be a novel source of the synthesis of zinc oxide nanoparticles and to detect the presence of hydrogen peroxide in various samples (10). Though, several macromorphological and pharmacological studies has been conducted for *Calliandra haematocephala,* detailed information of micromorphological characters like surface indumentum and trichome morphology is not well investigated. Present study was conducted to explore the structural diversity and distribution of trichomes, and vestiture types on the surfaces of all vegetative as well as reproductive parts to fill the void in information regarding micromorphology of *Calliandra haematocephala*.

Figure- 1. *Calliandra haematocephala* Hassk.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

Comment [F6]: Is this necessary?

Plant specimens were collected from campus of Govind Ballabh Pant University of Agriculture and Technology Pantnagar (India). Processing of plant specimens was done following standard taxonomic procedures (11). Plant species was identified with the help of relevant Floras and herbarium consultations at the herbaria of Botanical Survey of India, Northern Regional Center, Dehradun (BSD) and Forest Research Institute, Dehradun (DD). Voucher specimens were deposited in the herbarium of Department of Biological Sciences, Govind Ballabh Pant University of Agriculture and Technology Pantnagar, Uttarakhand, India. Fresh plants materials were used for vestiture and trichome study. Epidermal surfaces of each and every part were gently peeled off using razor blade. Peels were washed carefully and stained with aqueous safranin for 2-4 minutes. Stained epidermal peels were mounted in glycerine, covered and sealed with transparent nail polish to prevent dehydration. Prepared slides were examined under the light microscope in 4x, 10x, 40x, 100x objective and photomicrographs were obtained using Olympus MLX-B Plus microscope fitted with cell phone camera. Exact illustrations of trichome morphology were drawn by using prism type camera lucida at 10x magnification of objective lens. Trichomes were measured after appropriate calibrations using stage and ocular micrometer. For the description of vestiture, arrangement, position of trichomes on plant surfaces, shape and size standard taxonomic terminology given by Beentje (2010) and Payne (1978) with some required modifications was used.

Comment [F7]: Foliar epidermal features were studied and documented using the scraping method as described by some workers like Cutler (1978); Ogundipe and Olatunji (1991) and Nwokeocha (1996). The epidermal preparation of the adaxial and abaxial surfaces of the leaf blade were made for all accessions of *Andropogontectorum* and *Andropogongayanus* collected. The median part of well matured leaf samples were scraped using smooth sandpaper on a glass slide after which the peels were decolourized in 5% sodium hypoclorite (domestic bleach) for 30-60 minutes. The cleared epidermal peels were preserved in 50% ethanol, stained in Safranin O and counter-stained in Alcian blue to enhance contrast. All the preparations were mounted in 25% glycerol. Both adaxial and abaxial surfaces of the leaves were studied. Quantitatively, measurements were made for length and breadth of long and short cells, stomata and their area was calculated by multiplying the length and breadth of each character. Qualitatively, observations and descriptions of characters like type of stomata, subsidiary cells, trichomes, prickle hair, papillae were recorded. All microscopic measurements were taken with the aid of an ocular micrometer inserted in the eyepiece of the microscope. These measurements were later multiplied by ocular constant with respect to the power under which they were taken. Illustrations of the foliar epidermal features were photographed at X10 and X20 objectives underBK Series (Phase Contrast Microscope (PW-BK 5000T) equipped with a DCM 510 5 Megapixel Camera.

3. RESULTS AND DISCUSSION

Nine different types of trichomes were recorded- I) Glandular, multicellular, biseriate, colleter (MCO) which are 75-90 µm long, porrect, straight and recorded on peduncle and margin of petal (Figure-2.G1,G2,G3; Figure-3.A). II) Glandular, multicellular, uniseriate, brevicollate trichomes (MBR) which are 45-60 µm long, appressed, straight and recorded only on peduncle (Figure-2. J1, J2; Figure-3. B). III) Non-glandular, unicellular, uniseriate, attenuate trichomes(UAT) which are 75-600 µm long, straight and recorded on stem, leaflet margin (porrect), stipule, petiole, petiolule, rachis, abaxial surface of leaflet (spreading), adaxial surface of leaflet and bracteole (appressed) (Figure-2. A1, A2; Figure-3.C). IV) Non-glandular, unicellular, uniseriate, simple-acuminate (UAU) trichomes which are 120-225 µm long, straight and recorded on abaxial surface of leaflet (porrect), bracts and peduncle(oriented in different directions) (Figure-2.B1,B2; Figure-3. E). V) Non-glandular, unicellular, uniseriate, simple-muticose (UMU) trichomes which are 150-420 µm long, appressed and recorded only on peduncle (Figure-2. H1). VI) Non-glandular, multicellular, uniseriate, simple-subulate trichomes (MSU) which are 75-420 µm long, straight and recorded on peduncle (appressed) and abaxial surface and margin of petal (porrect) (Figure-2. C1,C2; Figure-3.D). VII) Non-glandular, multicellular, uniseriate, acuminate with cushion

trichomes (MCA) which are 480-600 µm long, arcuate, oriented in different directions and recorded only on peduncle (Figure-2.D1, D2; Figure-3.F). VIII) Non-glandular, unicellular, uniseriate, stalked-muticose trichomes (USM) which are 150-270 µm long, straight, appressed and recorded on peduncle (Figure-2. F1; Figure-3.G). IX) Non-glandular, unicellular, uniseriate, stalked-acuminate trichomes(USA) which are 180-270 µm long, tortuous, ascending and recorded on peduncle and bracts (Figure- 2. E1, E2; Figure-3.H).

Present study shows all vegetative and some reproductive parts of *Calliandra haematocephala* bear one or more different types of glandular and non-glandular trichomes (Figure 2 & 3). Two types of trichomes are glandular while seven types of trichomes are non-glandular in nature. Glandular trichomes were restricted to peduncle and petal margin in distribution but non-glandular trichomes were more common and recorded on most of the surfaces. Stem of *Calliandra haematocephala* bear only one type of trichomes (non-glandular, attenuate type) which were also recorded on other studied parts but their orientation on stem was porrect and the vestiture formed by such type of trichomes on surface was puberulent. The vestiture type recorded on both the surfaces of leaflets (pinna) was puberulent which do not show any taxonomic significance but two surfaces of leaflet can be differentiated on the basis of trichome observation as abaxial surface of leaflets has two different types of non-glandular trichomes with different orientations and adaxial surface of leaflets has only one type of trichome and their orientation was appressed. Rachis also bears same type of trichomes with similar orientation but forming lanate type of vestiture on surface. Eight types of trichomes were found present forming strigose type of vestiture on the surface of peduncle. The orientation of all types of trichomes on peduncle was found different. Along the margins and abaxial surface of petals show presence of glandular (colleters) and non-glandular (simple subulate) type of trichomes. Table-1 shows vestiture and trichome types on studied plant parts. El-Mary *et al*. (2003) have also reported non-glandular, unicellular tricomes and stalked glandular trichomes on stem and leaves of *Calliandra haematocephala* cultivated in Egypt. The present study, however, could not confirm the presence of glandular trichomes on leaves and petiole as these were recorded on only peduncle and petal margins. The complete structural diversity of trichomes (nine types) as observed in present work was not observed and reported by El-Mary *et al*. (2003). Shaheen *et al*. (2020) have also studieds leaves of 30 Fabaceae species from Pakistan which included *Calliandra bella* but the authors surprisingly realized very little variations in trichomes reporting generally non-glandular trichomes which are multicellular, uniseriate, unbranched, with bulbous base and pointed tips.

Comment [F8]: The result lacks clarity, it should follow a systematic order

Too wordy. Recast

The author should use the best terminology to explain the findings. An example of how i presented a resaerch paper can be seen below:

Abaxial surface (Plates 7A - D)
Anticlinal walls are straight and slightly wavy. Plate 7B

Long cells: rectangular, slightly elongated many times longer than broad; breadth more or less uniform; cell wall straight, prominently slightly wavy and sometimes straight; end walls mostly perpendicular; 4-5 rowed between the veins, cellwall is thick and straight, papillae present, numerous, small and of uniformsizes, present in between the stomata (Plate 7B). Cork cells (single not paired) are present (Plate 7C).

Short cells: solitary and more or less of equal sizes in pairs or in groups, in continuous rows along veins; mostly 1 row per costal region but occasionally up to 2 or 3, occurring on a straight line in between rows of stomata, found in between prickle hair as shown in Plate 7C.

Stomata: Fairly frequent to abundant; paracytic; subsidiary cells triangular to low-dome shape; end walls mostly perpendicular; 1-2 bands of stomata per intercostal zone (Plates 7C and D).

Interstomatal cells: Slightly rectangular; length and width more or less uniform; transverse and walls concave; anticlinal cell wall wavy. Plates 7C and D
Prickle hair: Frequent; oblique in shape, big round base with blunt end, in rows between the short cells just after the costal region. Plate 7C

Microhairs: Very frequent, longer than in the *Andropogon tectorum*, each hair has unique base surrounded by a cluster of cells surrounded by a cluster of cells that provide a bigger platform for the hair to sit; modified in shape; apex sharp to blunt; tapers up; thick cell wall as shown in Plate 7A. No glandular trichome present.

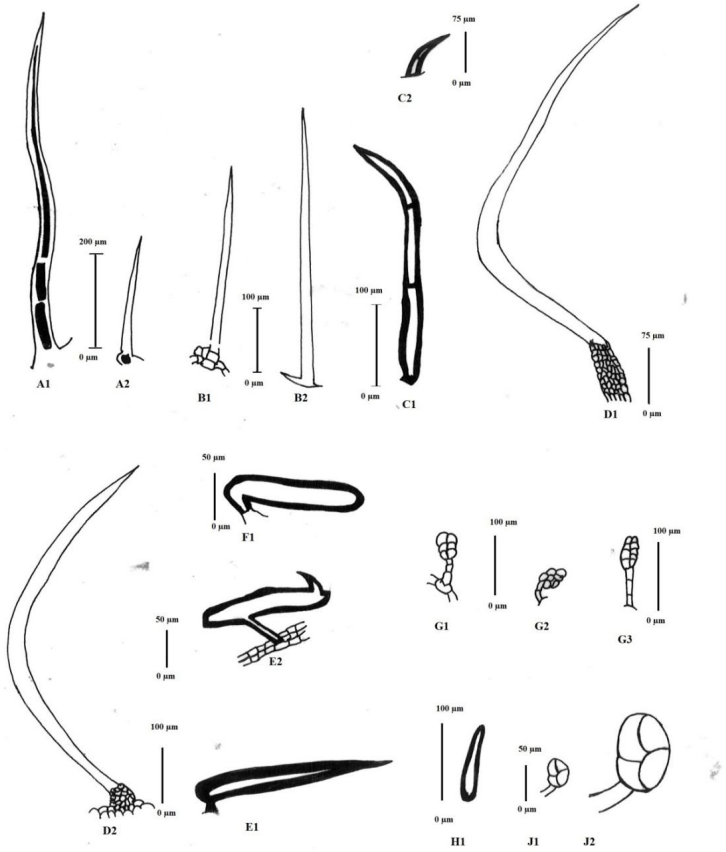

Figure-2. Structural diversity of trichomes in *Calliandra haematocephala*. A1, A2: Non-glandular, unicellular, uniseriate, attenuate; B1, B2: Non-glandular, unicellular, uniseriate, simple-acuminate; C1, C2: Non-glandular, multicelluar, uniseriate simple-subulate; D1, D2: Non-glandular, multicellular, uniseriate, acuminate with cushion; E1, E2: Non-glandular, unicellular, uniseriate, stalked, acuminate; F1: Non-glandular, unicellular, uniseriate, stalked, muticose; G1, G2, G3: Glandular, multicellular, biseriate, colleters; H1: Non-glandular, unicellular, uniseriate, simple-muticose; J1, J2: Glandular, multicellular, uniseriate, brevicollate.

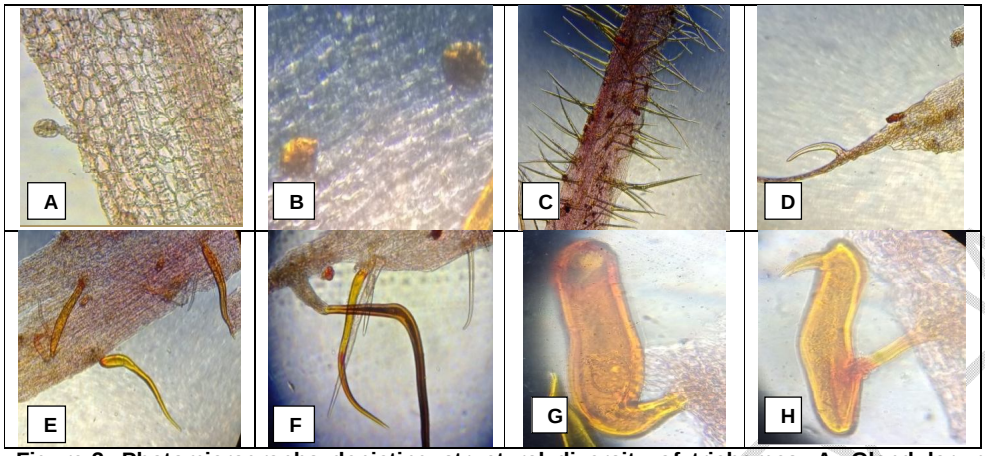

Figure-3. Photomicrographs depicting structural diversity of trichomes. A: Glandular, multicellular, biseriate, colleters; B: Glandular, multicellular, uniseriate, brevicollate; C: Non-glandular, unicellular, uniseriate, attenuate; D: Non-glandular, multicelluar, uniseriate, simple- subulate; E: Non-glandular, unicellular, uniseriate, simple-acuminate; F: Non-glandular, multicellular, uniseriate, acuminate with cushion; G:- Non-glandular, unicellular, uniseriate, stalked- muticose; H- Non-glandular, unicellular, uniseriate, stalked-acuminate.

Table 1. Vestiture type and types of trichomes in different studied parts.

Plant part	Vestiture type	Types of trichomes
Stem	Puberulent	Type-III
Stipule	Puberulent	Type-III
Petiole	Puberulent	Type-III
Petiolule	Puberulent	Type-III
Leaflet (Abaxial surface)	Puberulent	Type-III, IV
Leaflet (Adaxial surface)	Puberulent	Type-III
Leaflet (Margin)	Evenly distributed	Type-III
Rachis	Lanate	Type-III
Peduncle	Strigose	Type-I, II, IV,V,VI, VII, VIII, IX
Bracts	Puberulent	Type-III, IV, IX
Bracteole	Strigose	Type-III
Sepal (Abaxial surface)	Glabrous	
Petal (Abaxial surface and margin)	Puberulous & Puberulent	Type-I,VI
Filaments	Glabrous	
Anther	Glabrous	
Ovary	Glabrous	
Style	Glabrous	
Stigma	Glabrous	

4. CONCLUSION

The present study revealed the presence of nine different types of glandular (two types) and non-glandular (seven types) trichomes in *Calliandra haematocephala*. Peduncle was the part showing maximum structural diversity of trichomes by bearing eight different types of trichomes on its surface while filaments, anthers, ovary, style and stigma were completely glabrous. These micromorphological characters and vestiture types observed on the surfaces of different plant parts are diagnostic to distinguish and separate the species even when they are sterile or fragmentary.

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