International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 5, 2025
https://theaspd.com/index.php

Abiotic Elements Shaping Leaf Trichome Diversity In Plectranthus Amboinicus (Lour.) Spreng

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Abstract
Trichomes hold significance in leaves by minimising water loss, providing UV protection, inhibiting herbivores, aiding temperature regulation, and enhancing surface area. The morphological diversity and density of glandular and non-glandular trichomes on Plectranthus amboinicus obtained from five Southern India provinces were studied. Mature leaf samples of P. amboinicus were collected and their ecological parameters (soil and temperature properties) were recorded. The samples were examined under a light microscope to identify and quantify 13 types of trichomes (glandular and non-glandular). Population L-5 consistently recorded high trichome density. Non-glandular trichomes appeared more abundant, dense and pilose than glandular trichomes, with occurrence only on the abaxial leaf surface. Environmental factors influenced trichome variations, including elevated temperature, soil electrical conductivity properties, and pH. These ecological deviations are reflected in the anatomical insights of the species.
Keywords: *P. amboinicus, trichome, populations, glandular, non-glandular.*

INTRODUCTION

Plectranthus is a highly diverse genus in the Lamiaceae (Labiatae) family, belonging to the subfamily Nepetoideae, tribe Ocimeae, subtribe Plectranthinae, comprising about 300 species globally [10, 12, 19]. The genus is named from the Greek words 'plektron' (spur) and 'anthos' (flower), referring to the characteristic spurred flowers observed in most species. Owing to its aromatic nature, this genus is colloquially named "moth king". It is represented as one of the most prominent genera within the Lamiaceae, particularly prevalent in tropical Africa, Asia and Australia [2, 12]. The genus is distributed in all the habitats and altitudes of India, mostly in the Himalayas, Southern Ghats, and Nilgiris region. The most common species include P.amboinicus, P. barbatus, P. caninus, P. mollis, P. coetsa, and P. incanus – notable for their economic and restorative qualities [18]. The most significant aromatic restorative succulent plant, P. amboinicus (Lour) Spreng covered with short, delicate, erect hairs.

Trichomes are unicellular or multicellular appendages that arise from the aerial epidermis, characterised by distinguishing height by width proportion. They are the stalked projections: 'simple' or non-glandular (probably non-secreting) and `glandular-secreting' (GST) types. The latter regularly produce bountiful emissions to the plant surface, outside the cuticle, or stored within. Morphology of both of these trichome types, as found in various plants, fluctuates greatly [17]. Reports show that the sort and thickness of trichomes shift among species, due to different functional roles, environmental influence and evolutionary adaptations, creating changes in organs of the equivalent plant [16]. The synthesis of essential oil, storage and discharge in fragrant plants occur in various specific secretory structures, for example, ducts, oil cells, schizolysigenous and lysigenous depressions or glandular hairs, fitting in with their plant families, express that the types of trichomes or indumentum determine the qualities in higher plant taxa [5, 9]

Although multiple investigations exist on the phytochemical characteristics of P. amboinicus [8, 11], there is a limited understanding that exists in the morphology and thickness of glandular and non-glandular trichomes among the populations of this species. Until now, information concerning the morphology and intraspecific varieties of both glandular and non-glandular trichomes on the leaves of P. amboinicus remains unavailable, leaving a significant gap in our anatomical understanding of the species.

MATERIALS AND METHODS

In the current examination, the type and frequency of glandular and non-glandular trichomes were observed on both sides of five populations of P. amboinicus. These populations were collected from different provinces of South India and named L-1, L-2, L-3, L-4 and L-5. Each of the collected populations was observed to have unique features according to their ecological and phytogeographical conditions. Ecological parameters, namely average temperature, longitude, latitude, altitude, percentage of clay, silt and sand of soil and its texture, electrical conductivity and pH of the soil, were determined from each habitat (Table 1). The type and frequency of trichomes were investigated on five populations of P. amboinicus. From each population, mature leaves from five healthy stems were selected randomly for the examination of trichomes.

The pubescence level of adaxial and abaxial leaf surfaces was resolved under the binocular microscope based on the depiction given by ESAU. The leaf samples were utilised for microscopic investigations. Fresh leaves of each sample were fixed in an FAA solution (formalin 7.5%: acetic acid 7.5% and ethanol 85%) for 48 hours. Transverse hand sectioning of the lamina was produced using the mid-part of completely developed leaves, areas were then drenched in two subsequent treatments of 5% KOH (18 h for each treatment), the leaf samples were drenched in deionized water, treated for 2 minutes in glacial acetic acid, washed in deionized water, cleared in a bleach solution (5% sodium hypochlorite) and drenched in deionized water again. Leaves were dried out in a series of ethanol, stained using 1% carmine (in 99% water) for 45 minutes and successively stained with 0.01 methylene blue for 10 seconds and mounted onto microscopic slides for further examination [15].

Statistical analysis

The mean and standard deviation of the trichome were determined. One-way analysis of variance test (ANOVA) was used to locate the trichome number among the contemplated populations. SPSS version 16 was used as the virtual product for factual examinations.

RESULTS AND DISCUSSION

Mature leaf samples of P. amboinicus collected from five regions of South India were analysed for trichome distribution and density. The total number of trichomes on the leaf sections (adaxial and abaxial) was enumerated using the light microscope. The L-5 exhibited a greater number of trichomes on both the adaxial and abaxial surfaces with high soil pH (8.53) and low electrical conductivity (EC) (226 µS/cm) at high temperature. Similarly, the higher altitude (230 m). L-1 showed low trichome density with low pH and high EC. L-4 population reported moderate EC with a unique distribution of trichomes. L-2 and L-3 populations presented transitional trichome distribution. The results obtained through this study predict that pH and electrical conductivity have significant effects on the trichome morphology and structure.

Light microscopic observation of leaf samples helped to locate 13 types of trichomes on the leaves of P. amboinicus, which were classified into two main groups: Glandular and Non-Glandular (Simple). Glandular hairs comprised five types: peltate, digitate, short-stalked capitata, long-stalked capitata and sessile two-celled. Non-glandular trichomes included eight types: one-celled, two-celled, three-celled, four-celled, five-celled, six-celled, seven-celled and eight-celled. These findings revealed the distinct edaphic factors influencing the trichome characteristics.

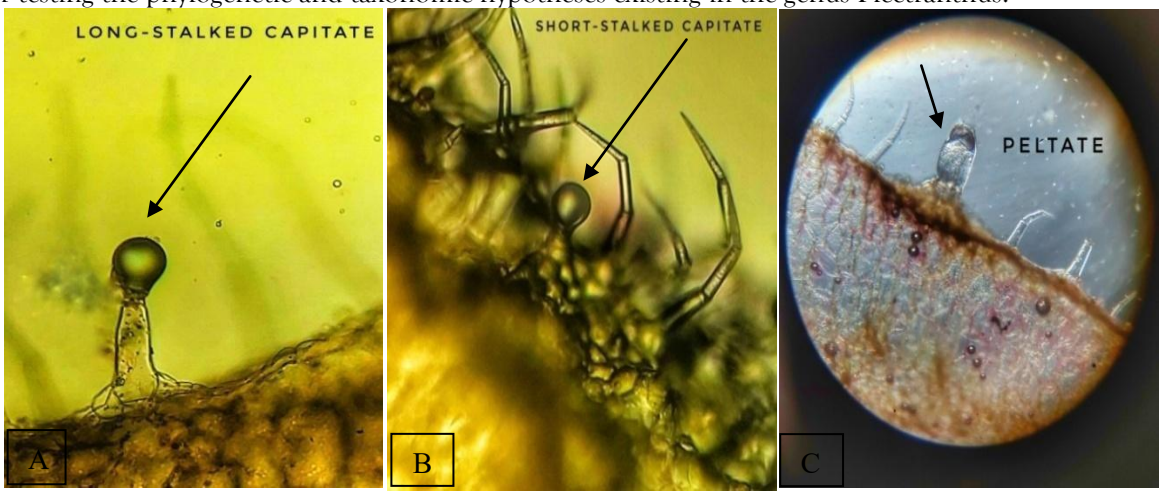
Table 1: Localities and edaphic factors of P.amboinicus populations.

Populations	**Localities**	**Longititude (E)**	**Latitude (N)**	**Altitude (m)**	**Temperature (°C)**	**Soil pH**	**Soil EC (µS)**	**Clay (%)**	**Silt (%)**	**Sand (%)**	**Soil texture**
Erode (L-1)	Karukampalayam Erode	77.717^0	11.341^0	183	31^0	8.17	508	16.666	26.666	56.666	Sandy Loam
Namakkal (L-2)	Nallampalayam, Tiruchengode	77.894^0	11.380^0	230	32^0	8.34	233	22.222	27.777	50	Sandy Clay Loam

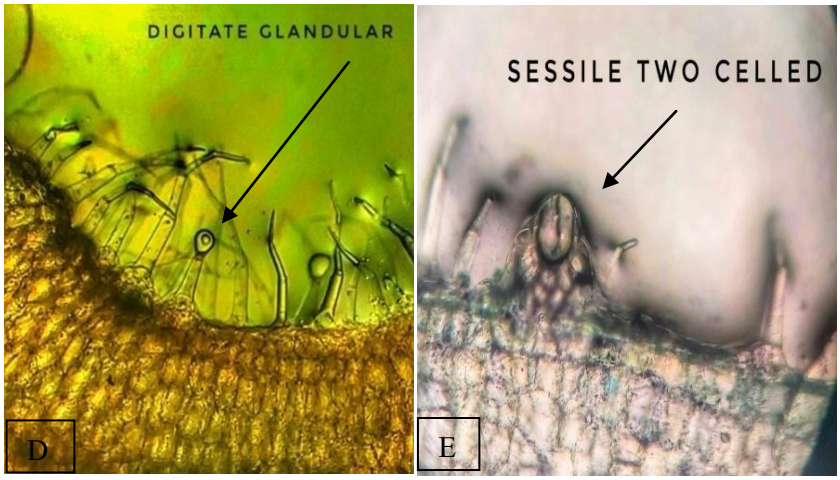
Karur (L-3)	Thoppur, Karur	77.842^0	10.989^0	101	31^0	8.32	328	16.666	26.666	56.666	Sandy Loam
Tirupur (L-4)	Avarangaadu, Nambiyur	77.320^0	11.358^0	301	31^0	8.21	228	42.105	18.421	39.473	Clay
Kozhikode (L-5)	Thalakkulathur, Kerala	75.780^0	11.258^0	13	34^0	8.53	226	10.810	27.027	62.162	Sandy Loam

Localities and edaphic parameters of Plectranthus amboinicus populations collected from five different sites in Southern India. Environmental data include GPS coordinates, elevation, temperature, pH, EC, and soil texture. Macronutrient composition is expressed in percentages of nitrogen (N), phosphorus (P), and potassium (K).

Presence of trichomes is a notable characteristic of Lamiaceae and its members. It is confirmed by the presence of a large number of trichomes on the epidermis of the aerial parts. Glandular trichomes are associated with specialised secretion of chemical compounds, involved in attracting animals. It offers chemical protection against creepy insects and bugs that may harm plant cells. Additionally, non-glandular trichomes are characterised by their ability to resist heat stress, expand resilience against cold, as a seed dispersal component, an extraordinary job in maintaining water balance in leaves and to redirect radiation [6]. The morphology of trichomes and thickness is a semi-variable trademark; it fluctuates under various ecological conditions [15]. This varied morphology of the trichome could prove as a useful source of data for testing the phylogenetic and taxonomic hypotheses existing in the genus Plectranthus.



Long–stalked capitate Short-stalked capitate Peltate



Digitate glandular Sessile two celled

Figure 1 (A–E): Glandular trichomes depicting structural diversity. (A) Long-stalked capitate, (B) Short-stalked capitate, (C) Peltate, (D) Digitate glandular, (E) Sessile two-celled.

Capitate glandular trichomes were categorised into two distinct types, long-stalked and short-stalked. Long stalked types in L-3 populations had few basal cells with one/two-celled heads, whereas in L-3 and L-5 samples, short stalked types with numerous basal cells were observed. Peltate trichomes appeared as short stalks and a broad head comprising of two cells. The highest number was found in L-4 and the lowest was in the leaves of L-3. Sessile hairs were found in two shapes, i.e. one-celled and two-celled, with a higher number of basal cells. Sessile two-celled trichomes had more basal cells with a clear partition in between. This kind had restricted distribution and was noticed only in the leaf samples of L-5. Digitate trichomes had long stalks and heads comprising differentiated cells. Digitate hairs were found abundantly in L-5, L-2 and L-3 populations. The least was observed in L-2 and the highest in L-5.

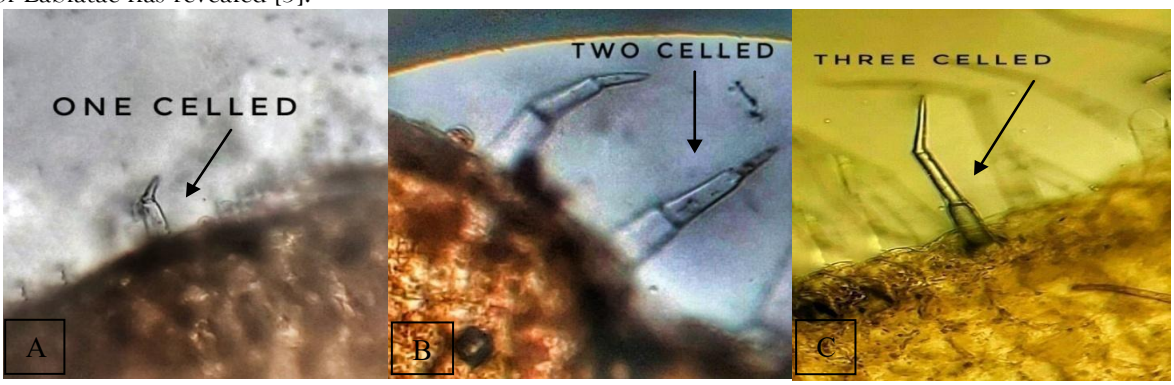
Table 2: Types of trichomes in the leaves of P. amboinicus

Province	**Erode**		**Kerala**		**Thiruchengode**		**Karur**		**Tirupur**	
Leaf surface	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abax
One celled***	0	0	380.0±7.0	406.0±3.6	0	0	0	0	0	0
Two celled**	189.0±7.9	181.7±10.4	192.0±4.4	201.3±6.8	35.3±1.5	43.3±1.5	21.7±2.5	36.3±2.3	40.3±6.4	52.0±6
Three celled***	15.3±2.1	21.0±1.7	0	0	201.7±4.2	212.3±6.1	230.3±2.5	244.0±5.6	178.7±10.4	191.0
Four celled***	13.3±1.5	13.7±1.5	0	0	22.7±3.2	26.3±3.5	22.7±2.5	40.3±6.4	29.3±5.5	54.0±6
Five celled***	0	0	0	133.7±5.0	250.0±3.6	254.7±14.3	108.0±4.6	122.7±5.5	0	0
Six celled***	0	0	0	43.0±4.0	0	0	0	0	0	0
Seven celled***	0	0	0	30.7±4.0	0	0	0	0	0	0
Eight celled***	0	0	0	19.3±3.2	0	0	3.3±0.6	3.3±0.6	8.3±3.1	8.7±
Peltate**	0	0	0	0	0	0	2.0±1.0	1.0±1.0	2.0±1.0	2.0±

Digitate***	0	0	0	11.3±2.1	2.3±0.6	4.7±1.5	3.7±1.5	4.3±1.2	0	0
Short stalked capitates***	0	0	0	2.3±2.1	0	0	1.7±0.6	2.7±0.6	0	0
Long stalked capitates***	0	0	0	0	0	0	2.0±1.0	2.0±1.0	0	0
Sessile two celled	0	0	1.0±1.0	0.3±0.6	0	0	0	0	0	0

Diversity and distribution of trichome types observed on adaxial and abaxial leaf surfaces of Plectranthus amboinicus across five Southern Indian populations. Values are expressed as mean ± standard deviation. Abbreviations: NG – Non-glandular, G – Glandular. Significance levels: *p < 0.05; **p < 0.01; ***p < 0.001.

Some confirmations of environmentally induced variation in the type and recurrence of leaf trichomes of Wigandia urens (Hydrophyllaceae) reported by [13], manifested higher trichome number in the dry than the wet season. Likewise, the plants exposed to daylight had more trichomes than those of plants which were concealed in shaded zones. The current examination of the leaves of P. amboinicus concedes different types of glandular discharging trichomes; however, the primary types remain peltate, digitate and capitate. The presence of capitate and peltate glandular hairs, in particular, is a characteristic highlight of Labiatae has revealed [3].



One celled Two celled Three celled

Four celled Five celled Six celled

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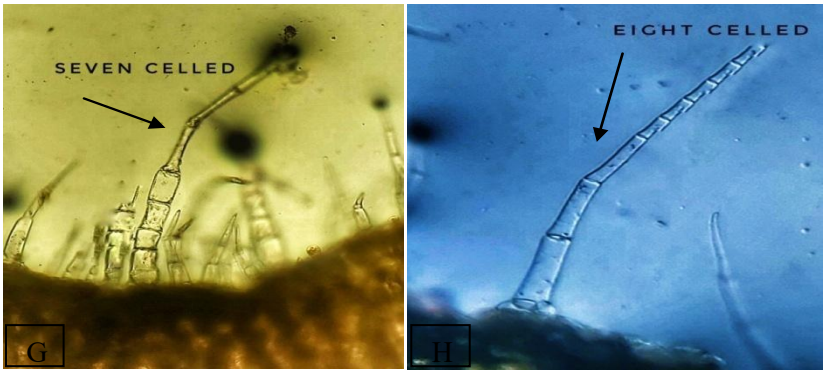

Seven celled Eight celled

Figure 2 (A–H): Non-glandular trichomes ranging from unicellular to multicellular forms. (A) One-celled, (B) Two-celled, (C) Three-celled, (D) Four-celled, (E) Five-celled, (F) Six-celled, (G) Seven-celled, (H) Eight-celled.

Non-glandular hairs were unbranched and the cells were linearly arranged with thin-walled cells. Higher numbers of one-celled trichomes were observed only in the leaves of L-5. Bi-celled or two-celled trichomes were observed least in the leaves of L-3 and highest on the leaves of L-5. Three-celled trichomes were higher in the leaves of L-3 and lower on the leaf surface of L-1. Four-celled trichomes were remarkably higher in L-4 and lowest in L-1 populations. Five-celled trichomes were marked high on the leaves of L-2 and least in the leaves of L-5. Six-celled trichomes and seven-celled trichome types had restricted distribution on the leaves of L-5. Eight-celled trichomes were higher in L-5 and lower in the populations of L-4.

Table 3: F value and Significance level

Trichome types	**F- value**	
	Adaxial	**Abaxial**
One celled	8841.3***	3804.4***
Two celled	857.077**	513.299**
Three celled	1307.3***	2235.3***
Four celled	39.297***	107.971***
Five celled	5353.3***	656.896***
Six celled	-	346.688***
Seven celled	-	172.735***
Eight celled	20.690***	49.433***
Peltate	9.000**	6.000**
Digitate	16.437***	40.458***
Short stalked capitates	25.000***	6.071***
Long stalked capitates	12.000***	12.000***
Sessile two celled	3.000ns	1.000ns

ANOVA-based F-values showing significance levels for variation in different trichome types between populations of Plectranthus amboinicus. Statistical significance is denoted as: ns – not significant, * – significant, ** – moderately significant, *** – highly significant.

The present examination shows varying trichomes among the population, which concurs with the observations of [15]. The distribution of both glandular and non-glandular trichomes not only differed between and within populations but also varied between the two surfaces of each leaf. The number of non-glandular trichomes was found to be higher than the glandular hairs. The latter were marked to be more on the abaxial side than the adaxial side. According to [6], the density of leaf trichomes in Tetradenia riparia decreased with leaf maturity. However, in the present study, the trichome density was found to be similar on the young and old leaves of P. amboinicus.

In our study, glandular and non-glandular trichomes were observed in the populations of L-5 and L-2 in a wider range. The reason could be attributed to the effect of temperature. The temperature of L-5 was recorded to be higher than the other localities, such as L-1, L-2, L-3 and L-4. As already specified by [13], the higher the light intensity, the greater the number of trichomes. Hence, this might be one of the reasons for variations in the number of trichomes in the leaf. The number of trichomes appeared lesser in areas such as L-4, L-1 and L-3 because the light intensity was recorded as lower when compared to L-5 and L-2. The localities L-5 and L-2 were noted as regions with a higher temperature than other localities. Also, the non-glandular trichomes appeared in higher numbers in L-5 and L-2. This correlates with the statement that non-glandular trichomes are involved in reducing heat stress, deflecting the radiation from sunlight, and maintaining water balance.

Studies agree with the report of [14], which states that phenotypic plasticity imparts significant effects on the morphology of plant structures. In the present study, phenotypic plasticity had significant effects on the trichomes collected from varied environments. Till date, the reports based on the effects of varied temperature and other soil characteristics on plants have been furnished by various authors. An attempt was made to compile the positive results that correlate with electrical conductivity and pH on foliar trichomes in their density and structure. In the current study, the electrical conductivity had a noteworthy impact on the trichome morphology and density. Comparative explanations support the above idea that the higher the EC level, the lower the yield of a plant [1]. The present investigation concludes that populations growing in high temperatures exhibit an increasing number of glandular and non-glandular trichomes.

CONCLUSION

The study demonstrated the interpopulation variation in trichome number, diversity and density in P. amboinicus collected from five different southern Indian regions. The tremendous density of non-glandular trichomes, especially in the population L-5, corresponded with high temperature, soil pH and lower electrical conductivity. Ecological conditions, specifically temperature and soil properties, impact trichome external development. Additionally, glandular trichomes, primarily found on the abaxial side, also vary between populations. These results may contribute to further taxonomic, anatomic and evolutionary, cultivation-related research on trichomes and their functional roles in the species.

Conflicts of interest: The authors declare that they have no conflicts of interest.

Ethics Statement: No fieldwork requiring specific permits was conducted. Plant material was collected responsibly without impacting local biodiversity.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial or profit sectors.

Acknowledgements: We are grateful to the Management of PSG College of Arts & Science and DST-FIST for providing the necessary infrastructural facilities.

Author's Contribution

All authors contributed to the study's conception and design. **Lekha Kumar** supervised the entire research project, providing guidance and expertise throughout. **Sangavi Jaganathan** conducted the research, including data collection on leaf trichome morphology in Plectranthus amboinicus. She also performed data analysis and interpretation. **Amjath Alikhan F** drafted the manuscript for the research article, encompassing the methodology, results, and discussion sections. All authors read and approved the final manuscript.

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