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MORPHOLOGY AND HISTOCHEMISTRY OF GLANDULAR TRICHOMES OF *TECTONA GRANDIS* LINN. F.

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ABSTRACT

Diversity, distribution and density of trichomes and glands of teak (*Tectona grandis* Linn. f.). 'Teak' a species is one of the most important timber trees of India. The objective of this study is to understand the morphology of the glandular trichomes and secretary products of glandular trichomes by using light, Ultra Violet and fluorescence microscopy. The chemical content was analyzed by using histochemical reagents. This morphoanatomical and histochemical study revealed that leaves of *T. grandis* possess four types of non-glandular and two type of glandular trichomes, with the latter differing both anatomically and in the composition of their secondary metabolites. Two classes of glandular trichomes were recognized as globular and reniform. A morphological study of the secretory head and the characterization of the secretory product are also presented. Secretory products of globular trichomes consisted of essential oils, where as reniform trichomes consisted basically of phenolic compounds such as flavonoids. Non-glandular trichomes were in different shape. Peltate and capitate glandular trichomes comprised one basal cell, one stalk cell and one head. The head of mature peltate glandular trichomes consisted of four-twelve secretory cells while that of the capitate glandular hairs was comprised of two cells. The fluorescent stain of peltate and capitate glandular trichomes indicated the possible presence of phenolic compounds.

KEYWORDS: Trichomes, Glands, Histochemistry, *Tectona grandis* linn.f., Secondary metabolites, Phenolic compounds.

INTRODUCTION

Teak (*Tectona grandis* Linn. f.) is belongs to verbinaceae. 'Teak' a species is one of the most important timber trees of India which is recognized for its physical and aesthetic qualities as one of the most important and valuable hardwoods in the world. [1] Teak is cultivated now throughout the tropics and has been used in traditional systems medicine in countries where it grows. Virtually every part of the teak tree has medicinal uses, and medical science has shown that the leaves have antibacterial, antiulcer and antifungal properties. Teak leaves are widely used in folk medicine as anti-inflammatory, analgesic, anti-ulcerogenic and healing agent. In Ayurveda the wood is considered a laxative, a sedative for the uterus, good for piles, dysentery and leucoderma. In folk medicine the roots are used for urinary tract problems, the flower for bronchitis, nausea and urinary tract problems too. The bark has been used to treat diabetes, and an extract of the bark has been found to have insulin resistance in mice. Plants used worldwide in traditional medicine have been studied for its biological activities and secondary metabolites used as active principles for drugs or as chemical models for the synthesis and semi-synthesis of drugs.[2-3]

Trichomes are generally derived from epidermal out growths on the surface of leaves or stems. [4] The different types of trichomes, such as hairs, spines and glands are related to their function in protecting the plant against insect or herbivore attack. [5] Specifically, glandular trichomes are known to secrete a wide of substances that are toxic to insects and/or cause allergic and irritant responses to herbivores.[6] On the other hand, trichomes also produce volatile compounds that attract pollinators.[7] As the plant products secreted by glandular trichomes may have industrial interests, attempts have been made to improve their production and/or to increase their yield by diverse breeding methods.[8]

The secretion production by these glands has shown high activity of proteases and acid phosphatases and has received attention due to the presence of passifloricins, insecticide-like alpha pyrones [9] and other phenolic substances able to inhibit the activity of metalloproteases involved in tumor invasion. [10] Epidermal structures like trichomes - their scanty distributions as well as poor development had been envisaged to be one of the major causes for high rate of transpiration leading to low rate of survival in case of micropropagated plants. [11] Trichomes are epidermal attachments of various shape, structure and function. They alter the boundary layer over surface, function in light piping, alter heat loss and aid in storage and secretion of secondary metabolites. [12] Glandular trichomes secrete various types of

compounds. A growing body of experimental evidence shows that terpene biosynthesis takes place within these trichomes.[13-14] Terpenes usually constitute the major lipophilic components of these secretions.

The present study aims at a detailed morphological analysis of the trichomes-their diversity, distribution and density of phenolics of teak plants. The aforesaid reason will probably help in understanding whether protection by both trichomes and phenols in the teak leaves is operational in tandem or there may exist any temporal or spatial difference between the protections rendered by the two (phenols and trichomes). We carried out an investigation of the morphology, distribution, and histochemistry of the glandular trichomes present on leaves of *T. grandis*, with the aim of clarifying their role. The objective of the present work was to characterize morphologically and histochemically the glandular trichomes, using techniques of light, ultra violet and fluorescence microscopy, seeking to establish relationships between trichome morphology and secretory product composition.

MATERIALS AND METHODS

Plant Material

Fresh young plant aerial parts of *T. grandis* were harvested in November (2013) from the Botanical Garden, PG and Research Department of Botany, Government Arts College, Dharmapuri, Tamil Nadu, India. From the seedlings as well as healthy mature trees about 25 years old plants were used as source of explants. For the purpose of these investigations, the term “mature leaves” refers to vegetative leaves harvested from mature trees, whilst “young leaves” describes leaves that had recently emerged from the seedlings were collected for the morphological and histochemical analyses.

Microscopy

Plant materials were used as fresh as well as cut into small pieces (5 mm2) in the laboratory and fixed in FAA (formalin-glacial acetic acid-70% ethanol, 5:5:90, v/v) for 48 h. the samples were observed with following histochemical reactions were carried out: Sudan red B [15] for lipids, periodic acid/Schiff (PAS) reagent [16] for polysaccharides with vicinal glycol groups, Ruthenium red [17] for pectins, mercuric bromophenol blue [18] for proteins, ferric trichloride and potassium dichromate [19] for phenolic compounds, hydrochloric vanillin [20] for tannins, Wagner and Dittmar reagent [21] for alkaloids, for terpenes containing steroids, sulphuric acid and 2-4-dinitrophenylhydrazine [22] for terpenoids. Standard control procedures were carried out simultaneously. [23] The observations were made in light microscope Nikon

Microphot - FXA. For the fluorescence microscopy investigation, fresh material was treated with: aluminium trichloride, magnesium acetate and lead neutral acetate [24] for flavonoids, and neutral red [25] for lipids. A epifluorescence microscope equipped with a UV filter (WU:330-385 nm), a dichroic mirror (400 nm) and a barrier filter (420 nm) were used. The autofluorescence investigation was carried out using the same instrument.

RESULTS

Trichome morphology and distribution of non-glandular and glandular trichomes were present on both the adaxial and abaxial leaf surfaces of teak (Figures. 1a-c). The non-glandular trichomes were distributed mainly on the veins and leaf margins and appeared more abundant on the abaxial leaf surface. They were were conical, unicellular, with thick and warty wall and calcified apical portion. In young leaves, the non-glandular trichomes partially obscured the glandular trichomes and it appeared that they matured at an early stage of leaf development (Figure 1a). Surrounding this trichome base were special subsidiary cells protruding above the level of the other epidermic cells. Four types of non-glandular trichomes were found: 1) short, with the sharp edge similar to spin, on the leaf adaxial surface and leaf margins (Figures 1a, 1c, 1g); 2) long, uniseriate with linear were present mainly on the major veins of the leaf abaxial surface (Figures 1j, 1k, 1l); 3) short, multicellular branched with sharp tips (Figure 1m) and 4) long, multiseriate with linear branched with sharp tips (Figure 1f, 1n).

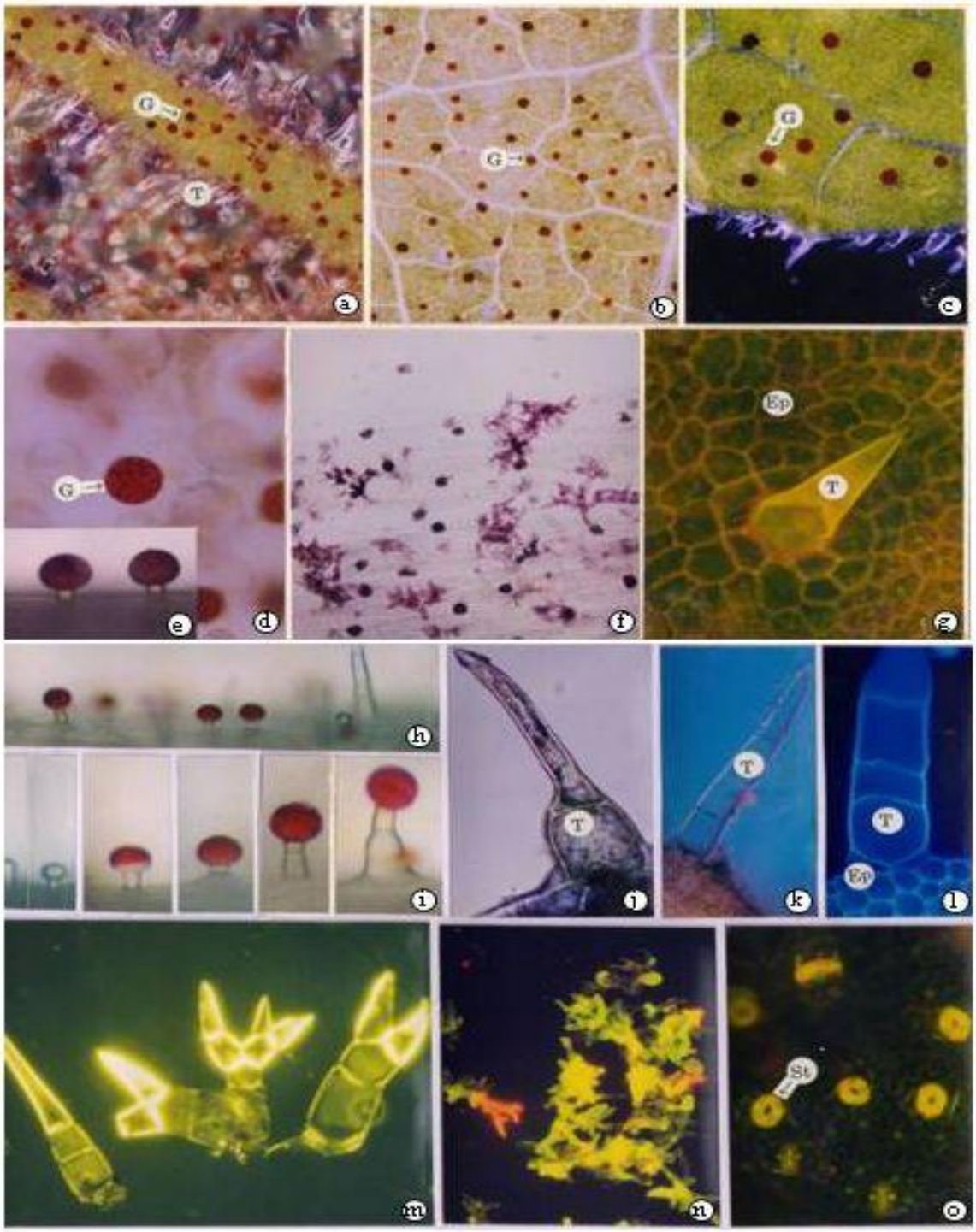
Glandular trichomes (Figures 1d, 1e) consisted of an epidermic basal cell, a uniseriate stalk and a unicellular secretory head. The stalk can be short, formed by a single cell, or long with up to four cells; and the upper cell showed a special type of differentiation, as a neck cell. The form and type of insertion of the secretory head allowed the distinction of two classes of glandular trichomes: 1) globular type (Figures 1d, 1e), with spherical secretory head, attached to the stalk by the usual way, regardless of the stalk cell number, and short neck cell; 2) reniform type (Figures 1h, 1i), with ellipsoidal head attached to the stalk by one end, when the stalk was short (just one cell), and when the stalk was long (2-four cells); neck cell long and curved. Globular and reniform glandular trichomes occured side by side on both leaf surfaces, being much more abundant on the abaxial surface. The short globular and reniform trichomes were more abundant and located in intervenal regions, whereas the long ones were scarcer and located along the veins, mainly on the abaxial surface. Globular and reniform trichomes with broken cuticle in the equatorial region of the secretory head cell were found

(Figure 1d). Globular and reniform trichomes were already present and fully differentiated at early stages of leaf development. On expanded mature leaves the glandular trichomes were still active.

Histochemistry of glandular trichomes-the results of histochemical tests for globular and reniform trichomes. Globular trichomes - The secretory product of globular trichomes was deep red in color, abundant, and located in the subcuticular chamber. Staining with Sudan red showed positive lipid reaction in both the head cells and neck cell walls (Figure 1d). Polysaccharides, as pectin, only occurred within protoplasts of secretory cells, but the secretory product had no reaction for this group of compounds with Ruthenium red, remaining clear. Multicellular branched trichomes showed blue autofluorescence under ultraviolet light (figure 1m, 1n). The presence of lipids was confirmed by the intense yellow fluorescence with neutral red fluorochrome. For the other histochemical tests, either under light or fluorescence microscopy, the reaction was negative or similar to the control. With neutral red fluorochrome, these trichomes showed intense gree fluorescence, indicating different chemical nature in comparison to globular trichomes, which fluoresce intense yellow under UV light. Flavonoids were identified with fluorochrome, fluorescing intense green under UV light, mainly when stained with aluminum chloride (Figure 1m-n). Stomata are present on both the adaxizl and abaxial surfaces but they are more abundant on abaxial surface. Each stomatal apparatus has kidney shaped guard cells as characteristic feature of dicot stomata (Figure 1o).

Figure-1. a. Microphotograph of adaxial surface of a juvenile leaf showing numerous reddish glands and trichomes (60 x). b. Adaxial surface of young leaf with reddish glands (60 x). c. Abaxial surface of leaf showing less number of glands and trichomes (150 x). d. Surface view of the apex of multicellular gland (300 x). e. Figure insert showing two multicellular secretory glands with very short stalk and filled with dye found on young leaves (300x). f. Multicellular branched trichomes from inflorescence stalk stained with safranin uniseriate trichome from the abaxial surface of the leaf (300x). g. a single unicellular trichome on the abaxial surface stained with acridine orange and excited with blue light (300 x). h-i. Various stages of development of secretory glands. Very young stage of gland has not accumulate any pigment. A single celled stalk is present. Before the division of the stalk cell, the head accumulates red dye (300x). j. Multicellular uniseriate trichome from the abaxial surface of the leaf stained with toludine blue (300x). k. Multicellular trichome present on the stem

surface in NDIC microscopy (300 x). l. Stem multicellular trichome stained with calcofluor white viewed with ultra violet light excitation (300 x). m. Multicellular trichome from mature leaf viewed between cross polarizes with green filter (300 x). n. Inflorescence multicellular trochome stained with acridine orange blue light excitation (150 x). o. Fluorescence microphotograph of stomata from the adaxial leaf surface stained with acridine orange with blue light excitation (600 x). (G = glands; T = trichome; Ep = epidermis; St = stomata)



DISCUSSION

Short unicellular hairs are characteristic of adaxial surface of the leaf. The basal epidermal cells that surround such hairs possess strongly fluorescent contents located in a centripetal position. The content might be siliceous in nature, since silica is known to accumulate in such a characteristic pattern in members of verbinaceae member such as *Lantana*. [26-27] The x-ray microanalysis will confirm the occurrence of silica and other mineral deposits in the leaf surfaces. In *T. grandis*, the development of basal epidermal cells was more evident in short non-glandular trichomes with appear on the leaf adaxial surface.

The morphology and the distribution of glandular trichomes in *C. verbenacea* were similar to those described for other species of the genus, [28] being always uniseriate, with unicellular secretory head and a typical shape. Despite of the variation in the number of stalk cells and in the size of the head cells, glandular trichomes could be classified as globular or reniform, according to the morphology of the head cell.

Structural lipids, probably cutin or suberin, were present on neck cell walls, as shown by the positive reaction with Sudan red B. This endodermal feature is commom in oil-secreting trichomes, which prevent backward-flow of the secreted material. [29] Neck cells with cutinized lateral walls were also found in species of Scrophulariaceae [30] and Lamiaceae, [31] although in peppermint (Lamiaceae) they were not described as cutinized, but as suberized walls. [32] Both glandular trichomes apparently accumulates their secretory product within the subcuticular space, as in glandular trichomes of Lamiaceae, [33] Scrophulariaceae, Asteraceae [34] and Bignoniaceae. [35]

The secretion of globular trichomes was oily and predominantly lipophilic, whereas reniform trichomes had hydrophilic secretion. The osmium tetroxide test indicated the presence of lipids in the composition of globular trichome secretion, with prevalence of unsaturated lipids, relatively large number of double bonds, and consequently low melting point, determining the fluidity of the secretion at room temperature. Although the osmium tetroxide test also indicated lipids in reniform trichome secretion, it should be considered that this is a broad-spectrum reagent, reacting not only with unsaturated lipids, but also with phenolic compounds, proteins and alkaloids, staining both secretion and protoplasts of secretory cells.

The difference in the autofluorescence pattern under light UV for the two types of trichomes also indicates differences in the secretory activity, with globular trichomes having blue

autofluorescence and reniform trichomes showing weak autofluorescence in the head. When globular trichomes were stained with neutral red, they showed an intense yellow fluorescence, whereas reniform trichomes showed blue fluorescence, confirming the different chemical nature of their secretion. The blue fluorescence of reniform trichomes can be a consequence of this fluorochrome reaction with structural lipids, such as, plasma membrane phospholipids, but not with the secretion itself.

Tectona grandis is a medicinal plant with versatile nature, apart from possessing high value of hardwood, itis also the unique source ofvarious types of compounds having diverse chemical structure. Several classes of phytochemicals like alkaloids, glycosides, saponins, steroids, flavonoids, proteins and carbohydrates have been reported in *Tectona grandis*. [36] Secondary metabolites such astectoquinone, 5-hydroxylapachol, tectol, betulinic acid, betulinic aldehyde, squalene, lapachol were extracted from the plant. [37-38] Several biological activities are attributed to essential oils, which were recognized as phytoalexins, insect antifeedant, feromones, defensive agents, allelochemicals or signaling molecules. Approximately 8,000 phenolic compounds were already identified in plants, having biological role generally related to antifungal, antibacterial and antifeedant activities of some structures. Although Ruthenium red indicated the presence of acid polysaccharides, as pectin, in both globular and reniform trichomes, this result must be analyzed with caution.

CONCLUSION

In conclusion, the chemical composition of the secretion of glandular trichomes in *T. grandis* is related to the morphology of the secretory head, regardless of its size and the stalk cell number. The secretion of globular trichomes consists mainly of essential oils, whereas the secretion of reniform trichomes consists basically of phenolic compounds, such as flavonoids.

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