



Gynostemium morphology and floral biology of *Thottea siliquosa* (Lam.) Ding Hou from the Western Ghats, India

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Abstract

Thottea is a medicinal under shrub belonging to the family Aristolochiaceae. Because of the presence of a very special gynostemium, the flowers of *Thottea* seem significant in floral ontogeny. Gynostemium is a composite floral component formed as a result of synorganization, and has a clear definition in many angiosperm groups, and has significant role in the species level systematics of *Thottea*. However, there exist conflicts among the scientist regarding the actual morphological and functional identity of gynostemium in *Thottea*. The present study deals with the morphology and floral biology of *Thottea siliquosa*, a medicinal shrub distributed in Peninsular India and Sree Lanka. The study also reports some interesting morphological variations within the floral construction of the plants located at the Ponnudi hills of the Western Ghats.

Keywords: floral biology; morphology; *Thottea siliquosa*; Western Ghats

Introduction

The medicinal genus *Thottea* belongs to the family Aristolochiaceae and comprises 45 species in Asia (Yao, 2015; Mustaqim and Putra, 2020) [17, 20]. The flowers are actinomorphic and trimerous with a synorganized reproductive component called gynostemium. It is a fusion product of male and female reproductive organs. However, in the gynostemium of the genus *Thottea*, the fusion between the floral parts is incomplete (Shaiju and Omanakumari, 2010). Along with other morphological characters, gynostemium is also considered significantly in the taxonomic characterization of *Thottea* spp. The gynostemium in *Thottea* towards its upper part is seen split forming a number of lobes. Only few studies were conducted with regard to the micro morphology and floral biology of the gynostemium in *Thottea* and the authors differently identified the functionality of the lobes as 'pollen capturing structures', 'stylar lobes', or 'stigmatic lobes' (Leins and Erbar, 1988; Shaiju and Omanakumari, 2010; Yao, 2013; Robi *et al.*, 2014) [16, 12]. This confusion makes an urgency to characterise the micro morphology and functional parameters of this taxonomically significant structure.

Materials and Methods

Study area

T. siliquosa (Fig. 1A) was collected from different regions of Kerala such as Ponnudi hills of Thiruvananthapuram district (Voucher No. TBGT 95922, GPS coordinates: 8.73°N & 77.12°E), Palode of Thiruvananthapuram district (Voucher No. TBGT 95930, GPS coordinates: 8.75°N and 77.02°E) and Kulathupuzha of Kollam district (Voucher No. TBGT 95931, GPS coordinates: 8.92° N and 77.03° E). The voucher specimens were deposited at the herbarium of

Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode, Thiruvananthapuram, Kerala.

Micro morphological studies

Morphological parameters were assessed using stereo zoom microscopy and Scanning Electron Microscopy. Fresh flowers were used for stereo zoom microscopy (LEICA EZ4 HD, Switzerland). Mature flowers fixed in FAA were used for SEM studies. After fixation, they were subjected to sequential dehydration (50-100% ethanol), drying, mounting on metallic stubs and coating with gold film in a sputter chamber and then observed with a scanning electron microscope (ZEISS EVO 18 Research microscope, Germany).

Floral biological studies

Pollen grains collected at different time intervals were subjected to pollen fertility by Acetocarmine staining method (Shivanna and Tandon, 2014) [15]. For pollen viability studies, FCR test (Heslop-Harrison and Heslop-Harrison, 1970) was conducted and the percentage of viability was calculated by making observations with a trinocular fluorescent research microscope (Nikon Eclipse Ci epifluorescence microscope, Japan). *In vitro* pollen germination experiment was carried out using Brewbaker and Kwack's medium (Brewbaker and Kwack, 1963) [2] supplemented with different sucrose concentrations (0.03-50%). Peroxidase activity test was performed to find out the peak receptive time of stigma (Kearns and Inouye, 1993) [6]. Receptive area on the gynostemium was identified by cytochemical localization of stigma-surface esterases (Mattsson *et al.*, 1974) [8]. *In vivo* pollen germination was investigated by observing pollen germination and pollen tube entry into the stigma in manually pollinated flowers

using Aniline blue fluorescence microscopic method (Shivanna and Rangaswamy, 1992) [14]. The slides prepared were observed under a trinocular fluorescent research microscope (ZEISS Axio Observer.A1, Germany).

Results and Discussion

The members of the family Aristolochiaceae have very interesting floral characteristic features, which attract the attention of Botanists (Nair and Narayanan, 1962) [10]. Without any exception, the flowers of the genus *Thottea* are also unique and with some interesting features. The flowers of *T. siliquosa* are trimerous having deep purple-coloured tepals with acute tips. The tepals are free and pubescent. Flowers with four tepals were rarely observed (Fig 1B).

Morphology of gynostemium

The stereomicroscopic studies revealed the external morphology of the gynostemium. There is an incomplete fusion with regard to the reproductive parts in the gynostemium (Fig. 1C). There are three bundles with three extrorse stamens in each. Very rarely, flowers with four bundles of two stamens were observed in flowers with four tepals. 4+3+3, 4+4+4, 2+2+2, 3+3+3+2, 3+2+1+1 and 3+3 stamen patterns were also noticed. Interesting variations in stamen number and pattern were reported earlier in *T. duchartrei* (Nazarudeen and Sabu, 2002; Shaiju and Omanakumari, 2010).

The stamen bundles of *T. siliquosa* were surrounded with numerous multicellular uncinata hairs. Very rarely, solitary appendages with pointed terminal regions were observed just below the bases of the styler lobes in alternate position to the stamen bundles (Fig. 1D). These appendages were similar in colour to the filaments of the stamens. They were one or two in number per flower. These appendages were also covered with a large number of uncinata hairs all over them. These appendages did not produce any exudation at the time of stigma receptivity. Thus, morphological studies indicate that these rare appendages show similarity with the stamens.

All the collected plant samples showed three kinds of gynostemium on the basis of colour of the styler lobes. The first type was the one in which the entire styler lobe was reddish brown in colour (Fig. 1C). The second type was with pale yellow colour towards the base of styler lobes and at the central region where the bases of styler lobes meet and reddish-brown towards the terminal part (Fig. 1E). The third type was with pale yellow-coloured styler lobes in which the central region where their bases join is also pale yellowish in colour (Fig. 1F).

The intensity of the reddishness varies in different flowers. The terminal part of the gynostemium is split into five to nine, mostly seven styler lobes with tapering ends. Some of the styler lobes are bifurcated at their tips. Very rare occurrence of trifurcate styler lobes was also noticed (Fig. 1G).

These lobes are found spreading, wet and fleshy and during the receptive stage (Fig. 1C) and during the non-receptive stage, they become closed and shriveled (Fig. 2A). The

reddish brown colour of the styler lobes is due to the presence of very small red spots in patches (Fig. 2B). The presence and variation in the distribution of these patches makes the styler lobes appear in different colour combinations as explained earlier. Below the styler lobes, larger reddish-brown spots were seen surrounding the styler column (Fig. 2C). The styler column and styler lobes were covered with uncinata hairs. The styler lobes were covered with uncinata hairs either entirely or towards their terminal regions. The styler lobes of the flowers collected from the Ponmudi hills showed the presence of uniseriate filamentous hairs with elongated cells on their apex (Fig. 2D). These hairs were similar to the filamentous hairs on the inner surface of the perianth lobes (Fig. 2E). The scanning electron microscopic analysis provided a detailed picture on the micro morphology of the styler lobes (Fig. 2F). In *T. siliquosa*, the styler lobe has an irregular surface with ridges, furrows and a waxy coating. However, any distinguishable stigmatic structure or area was unable to be detected.

Floral biology

Reproductive traits in plants can influence the operations of micro evolutions and forms of macro evolutions (Barrett, 2008).

The potentiality of pollen grains can be expressed by their fertility, viability, germinability etc (Dafni and Firmage, 2000). Pollen grains showed the highest fertility percentage of $95 \pm 0.22\%$ (Fig. 3A and Fig. 4) and the highest viability percentage of $93.5 \pm 0.35\%$ (Fig. 3B and Fig. 4) when collected between 10:00 am and 12:00 pm. Pollen grains collected between 11:00 and 1:00 pm performed the highest rate of germination and it was $51.16 \pm 0.64\%$ (Fig. 3C and Fig. 4).

Like pollen viability, stigma receptivity is also essential for accomplishing pollination and seed formation (He *et al.*, 2017). Stigma receptivity was maximum when checked between 9:00 am and 1:00 pm (Fig. 3D).

After 2:00 pm, a sharp decrease in receptivity was noticed. On the cytochemical localization of stigma-surface esterases, the entire styler lobe became reddish in colour indicating the location of stigmatic tissues (Fig. 3E). However, positive indication of receptivity was absent on the appendage on the gynostemium. Aniline blue fluorescence microscopic method revealed pollen germination on the entire surface of the styler lobes (Fig. 3F).

The pollen grains failed to germinate on the appendage and on the filamentous hairs. Thus, cytochemical localization of stigma-surface esterases and Aniline blue fluorescence microscopic method for *in vivo* pollen germination revealed the presence of stigmatic tissue on the whole styler lobe. Based on floral anatomical studies, Renuka and Swarupandan (1986) revealed the presence of staminodes in *Bragantia dalzellii* and *B. wallichii*. The reproductive biological studies on *T. siliquosa* indicate that the rare appendages do not show any stigmatic receptivity.

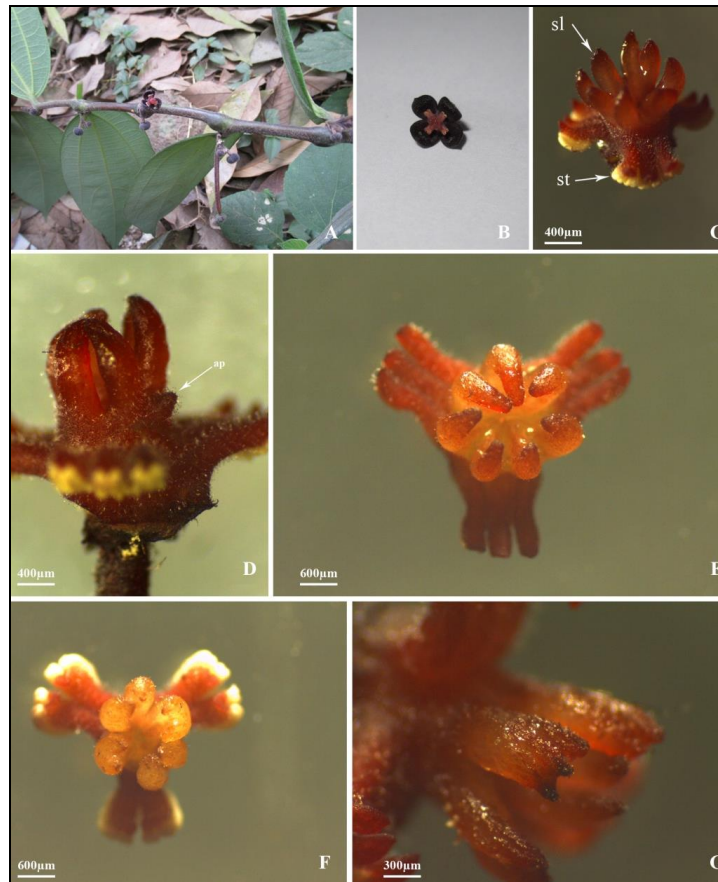


Fig 1: Habit and morphology of gynostemium in *T. siliquosa* (A) Habit with flowers; (B) Flower with four tepals and four stamen bundles; (C) Gynostemium (receptive stage) sl: stylar lobe, st: stamen (D) Gynostemium with an appendage (ap); (E) Gynostemium having stylar lobes with reddish brown distal regions and pale yellow basal regions; (F) Gynostemium with pale yellow stylar lobes; (G) Trifurcate stylar lobe.

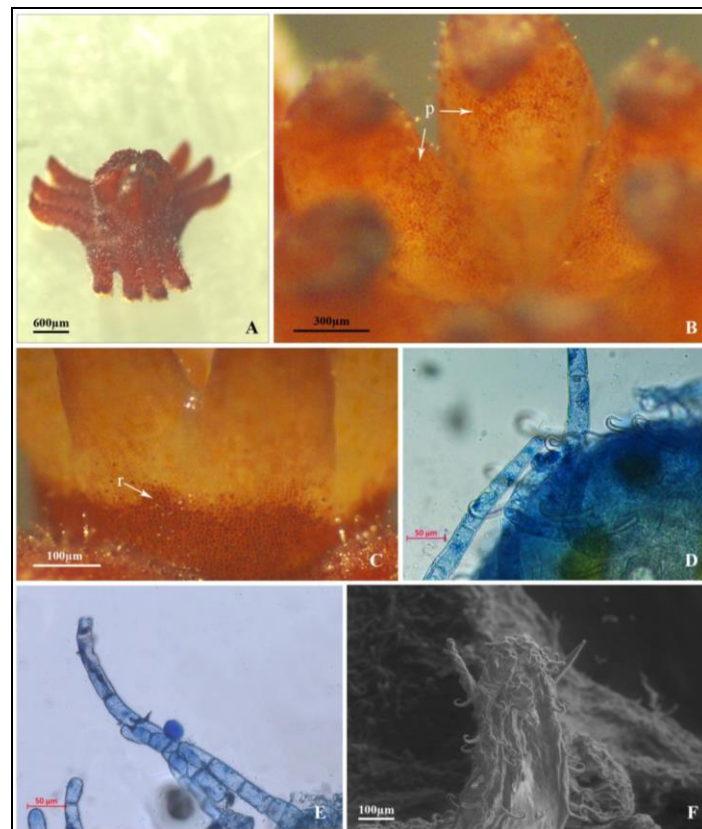


Fig 2: Gynostemium morphology of *T. siliquosa* (A) Non receptive gynostemium (B) Stylar lobes showing patches of red spots (p); (C) Gynostemium showing red spots (r) on the stylar column; (D) Filamentous hairs on stylar lobe; (E) Filamentous hairs on perianth; (F) SEM of stylar lobe.

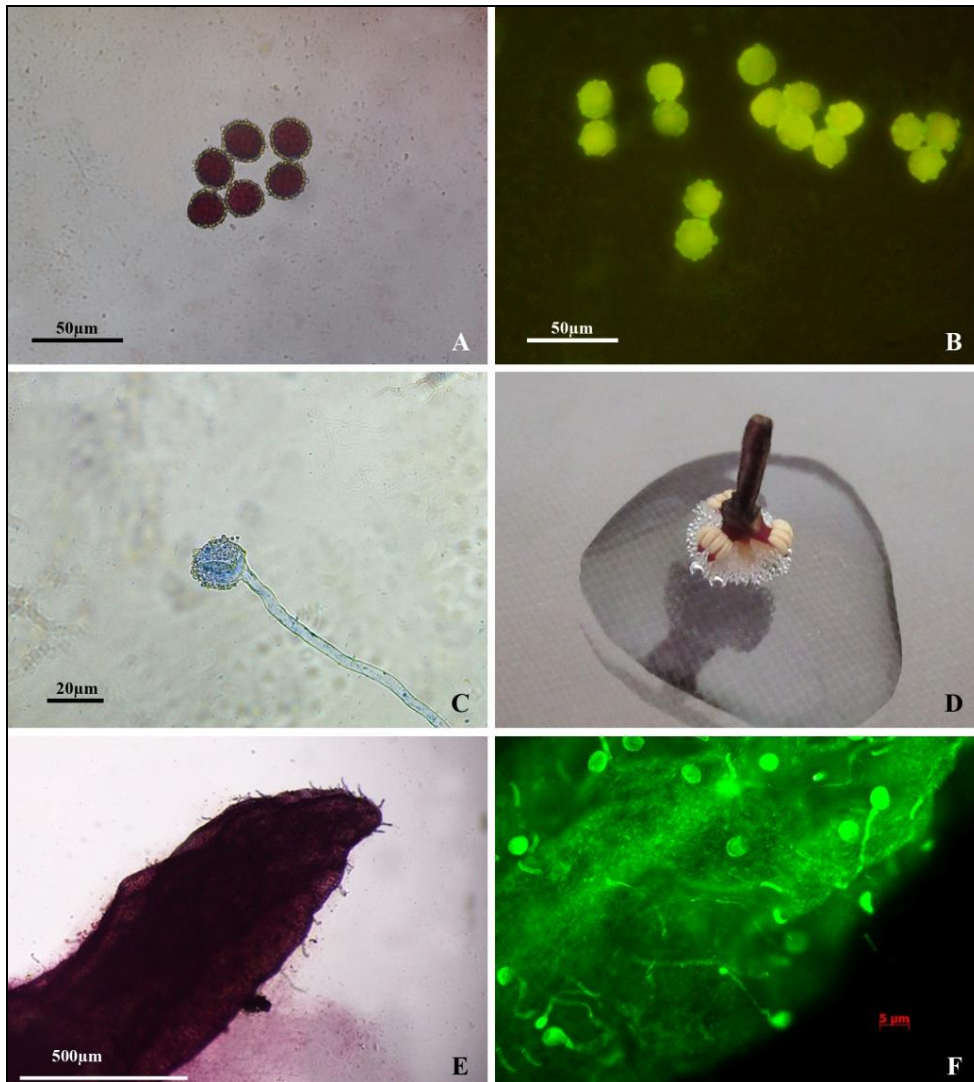


Fig 3: Reproductive biological studies on *T. siliquosa* (A) Fertile pollen grains after Acetocarmine staining; (B) Viable pollen grains showing fluorescence up on FDA staining; (C) Pollen germination (*in vitro*); (D) Peroxidase activity test - Release of Oxygen bubble from receptive area; (E) Cytochemical localization of stigma specific esterases showing the receptive area in red colour; (F) Aniline blue fluorescence – *in vivo* pollen germination.

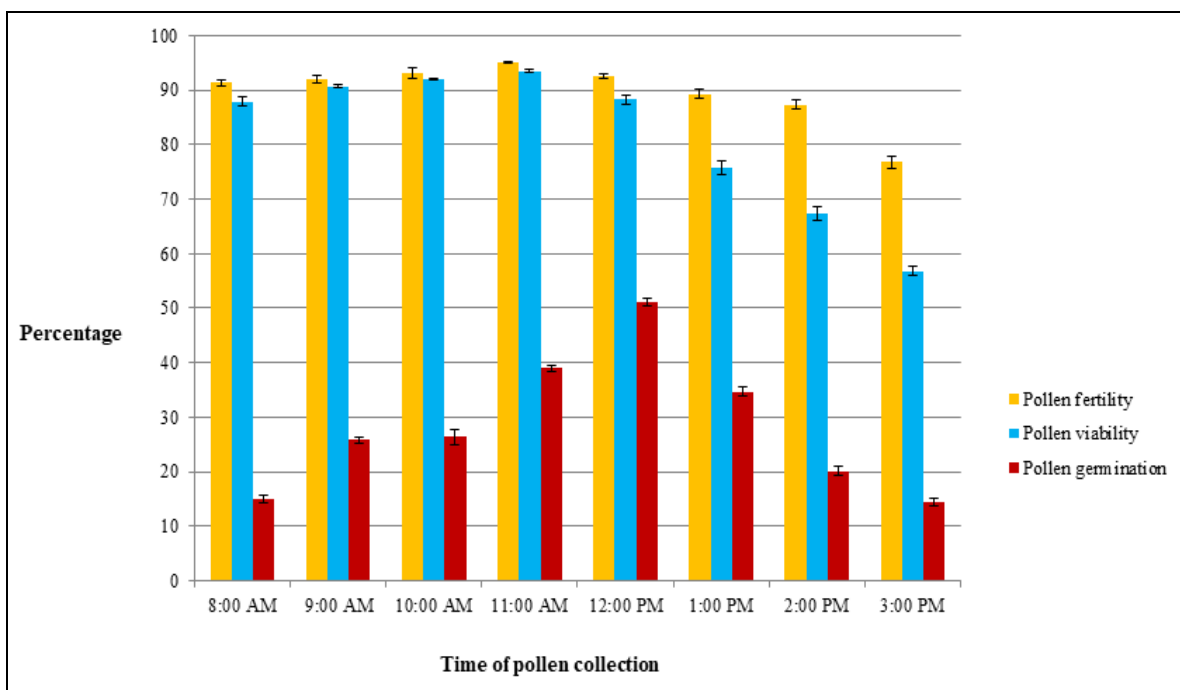


Fig 4: Pollen fertility, pollen viability and *In vitro* pollen germination studies in *T. siliquosa*

Conclusion

Gynostemium in *T. siliquosa* shows an incomplete fusion of the male and female reproductive structures. Morphological studies involving stereo zoom microscopy and SEM analysis could not identify any special stigmatic structure on the stylar lobes. However, the reproductive biological indices revealed that the entire stylar lobes form the stigmatic tissue regardless of the location of the uncinata hairs. Both the morphological and reproductive biological studies confirmed the morphological status of the appendages as staminodes.

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