ONTOGENY OF FOLIAR STOMATA AND TRICHOMES IN MULBERRY (*MORUS* L.)

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THE structure and development of stomata in several plant species have been studied by various workers[1–7]. Stomatal frequency and the size of a few varieties of mulberry have earlier been reported[8]. The development of stomata and trichomes of the genus *Morus* L. is unknown. The present communication describes the ontogeny of these structures in a few cultivated species of the genus, viz. *M. alba* L. (varieties: K2 and Local), *M. multicaulis* P. (variety Kosen) and *M. laevigata* Wall. Fresh (young as well as mature) leaves were collected from the field at CSR&TI, Mysore. Epidermal impressions of mature leaves obtained on a thin film of quickfix were mainly studied for stomatal structures. Peelings obtained directly from young leaves and mounted in acetocarmine were used for developmental study.

Leaves are hypostomatic and the stomata are anomocytic in all the species. Based on the size, they can be classified into two types in all the varieties. While most of the stomata are normal, with the size varying from 14 to 16 μm × 11 to 12 μm, a few stomata are found to be exceptionally large with the size varying from 22 to 32 μm (av. 25.7) × 15 to 21 μm (av. 17.3 μm). Such large stomata were earlier reported only in certain species of *Brosimum* SW and *Ficus* L. of the family Moraceae[9]. Stomata, when present above the veinlets are usually of the larger type. While normal stomata are usually surrounded by 4 or 5 neighbouring cells, the "giant stomata" are seen surrounded by 7–9 cells. Cuticular striations are present in all the epidermal cells on the lower surface (figure 3).

The stomatal meristemoids at various stages of development are small in size with dense granular contents, rectangular/polygonal in shape and are irregularly distributed among the epidermal cells (figure 1).

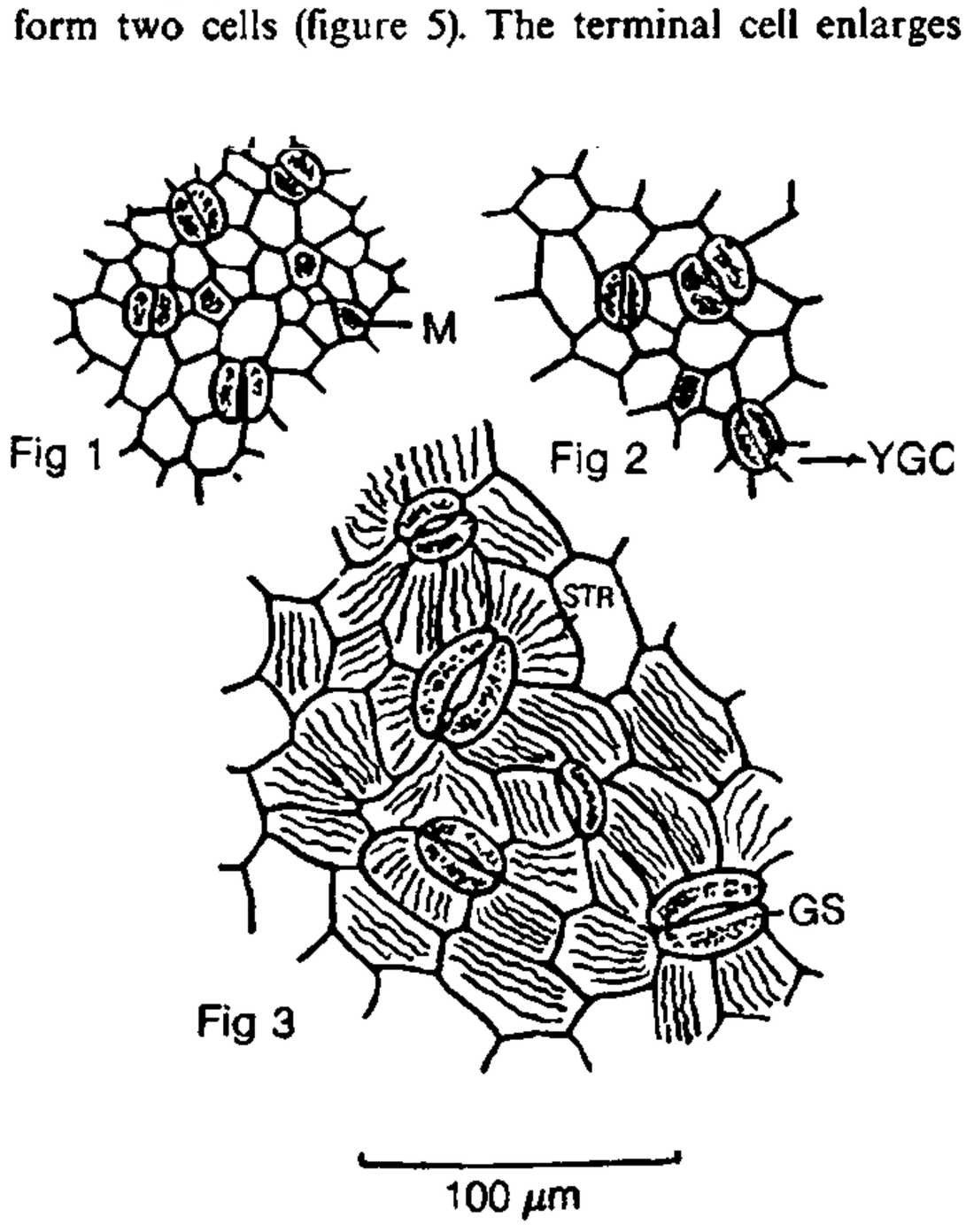
The ontogeny of stomata in all the species follows the same pattern. The stomatal meristemoids directly function as the guard cell mother cell which enlarges and undergoes a vertical division forming two small cells (figure 2). These cells assume lenticular shape, develop an intervening pore and form two bean-shaped guard cells. As the stomatal meristemoid functions directly as the guard cell mother cell, the development is perigenous.

Both glandular and non-glandular trichomes are present in *Morus*. The glandular trichomes are of varying sizes and shapes. They consist of 1- or 2-celled bases and multicellular biseriate heads. The head usually consists of 4 or 6 cells arranged variously (figures 13–16). Glandular trichomes with an 8- or 16-celled head, as reported in the floral parts of *M. alba* (var. Kanva-2)[10] were not observed in the leaves of any of the varieties investigated by us.

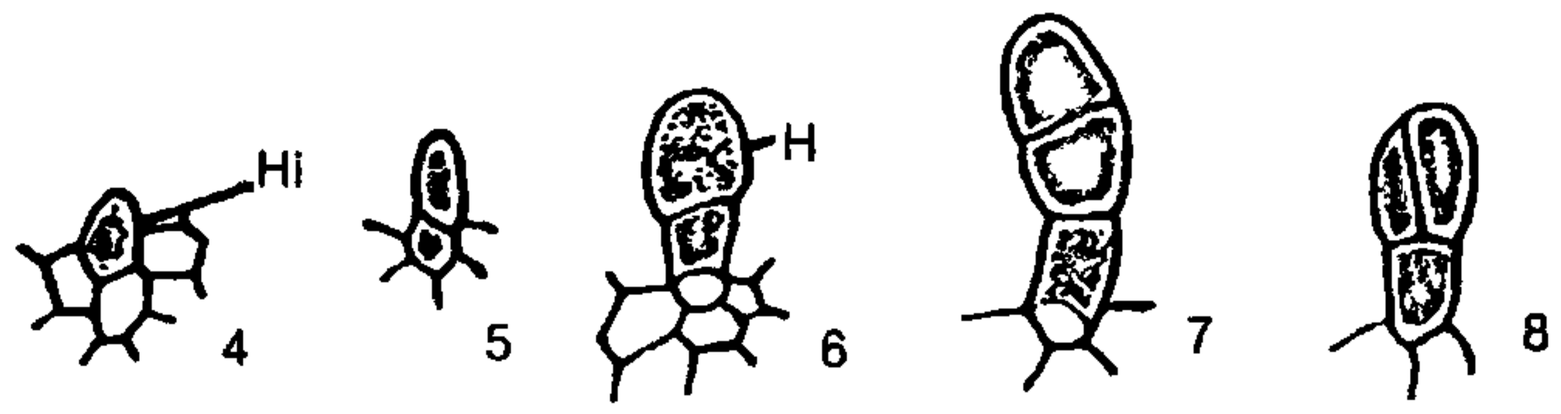
Non-glandular trichomes are of varying lengths and are unicellular, thick-walled. They have a bulbous base and an elongated tapering end which may be curved (figure 18).

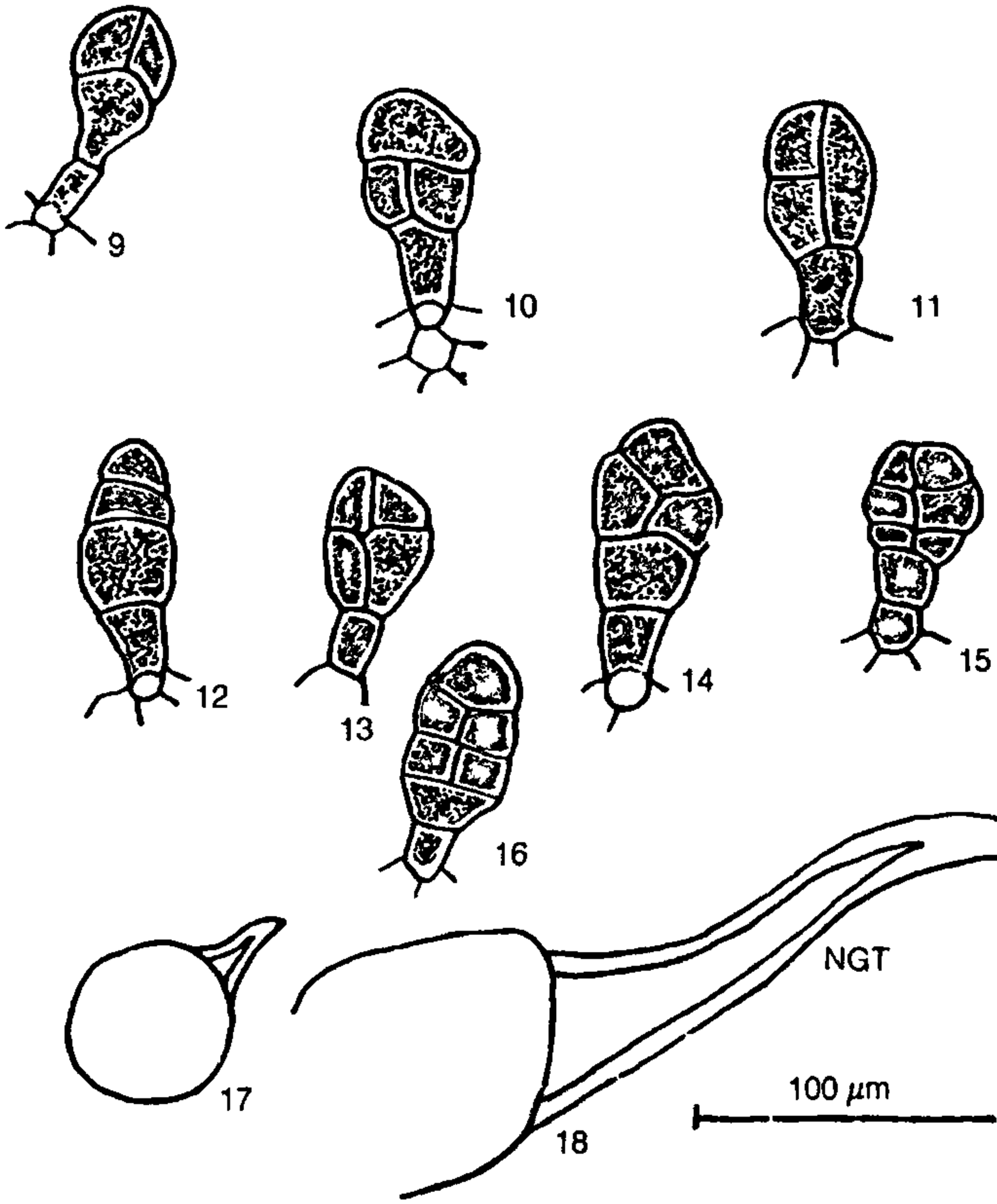
Glandular and non-glandular trichomes develop from single protoderm cell which cannot be distinguished in their initial stages from stomatal meristemoids. The hair initial soon becomes papillate (figure 4) and then elongate to form the single-celled non-glandular trichome (figures 17 and 18). The development of the glandular trichome is as follows:

The hair initials (Hi) of glandular trichomes become papillate but divide by a transverse wall to form two cells (figure 5). The terminal cell enlarges



Figures 1–3. Development of stomata in *Morus*. [GS, Giant stomata; M, Stomatal meristemoid; STR, Striations; YGC, Young guard cells.]





Figures 4–18. Development of trichomes in *Morus*. **4–16.** Development of glandular trichomes; **17 and 18.** Non-glandular trichomes. [Hi, Hair initial; H, Head initial; NGT, Non-glandular trichome.]

(figure 6) and divides to form a 4- or 6-celled head (figures 7–16). The first division of this terminal cell (H) is usually transverse and forms a uniseriate, two-celled head (figure 7) but rarely divides vertically to form a biseriate two-celled head (figure 8). In *M. laevigata* the initial head cell undergoes two successive transverse divisions to form a uniseriate three-celled head (figure 12) which in turn divides vertically to form a six-celled head (figure 15).

Thus all glandular and non-glandular trichomes on the leaves of mulberry start their development from single protoderm initials but the later development of glandular trichomes does not follow a uniform pattern.

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BIOCHEMICAL CHANGES DURING POST-HARVEST STORAGE OF BUTTON MUSHROOM (*AGARICUS BISPORUS*)

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WHITE button mushrooms (*Agaricus bisporus*) are harvested immature (button stage) and have a very short shelf-life. Post-harvest changes resembling growth and maturation cause weight loss, veil opening and external browning. This results not only in low consumer preference[1] but also a loss in nutritive value[2]. Extending the shelf-life of mushrooms is of economic importance and basic studies on post-harvest biochemical changes are required to evolve sound post-harvest practices. Since storage temperature has a profound effect on shelf-life of the mushrooms[3] its effect on protein, amino acid, sugar and phenol content of the mushroom was studied in the present investigation. The results of this analysis necessitated a study of the activities of protease (EC 3.4.22.1) and polyphenol oxidase (PPO, EC 1.10.3.1).

Button mushrooms (3.5 ± 0.2 cm cap diameter) with differentiated but non-stretched velum were harvested, cleaned and packed (200 g) in 100-gauge-thick and perforated (0.5% vent area) polyethylene bags. These were stored at 5°C and 85–90% RH, 10°C and 70–75% RH, and 15°C and 55–60% RH. Chemical composition and protease activity were determined daily till the 4th day.

The protein content was determined by the method of Lowry *et al*[4]. The samples were exhaustively extracted in 80% ethanol for determination of phenols[5], amino acids[6] and sugars. Total soluble sugar and reducing sugars were determined by the anthrone and Nelson–Somogyi methods[7] respectively. Values for non-reducing sugars were derived by the difference. The enzymes were extracted in chilled phosphate buffer (0.1 M, pH 6.8) in a Virtis-type micro-tissue homogenizer. The homogenate was centrifuged at 3000 *g* for 20 min in a refrigerated centrifuge and the supernatant was used as enzyme source. Protease was assayed[8] with haemoglobin as substrate. The reaction mixture for PPO contained, in a final volume of 3 ml, 2 ml of 0.1 m phosphate buffer, (pH 6), 5 μmoles catechol and suitably diluted enzyme source. The change in absorbance was followed at 495 nm in a Spectronic-21 (Bausch and Lomb) colorimeter. The activity of PPO was recorded at 25°C and also at the storage temperatures, i.e. 5, 10 and 15°C, after 96 h of storage.

The chemical composition data are shown in table 1. The decrease in total sugars during storage and at higher temperatures was due largely to a decrease in non-reducing sugars than to decrease in reducing sugars. This may be because, during post-harvest growth, maturation and gill development, the respiratory rate of mushrooms increases and trehalose, the most abundant non-reducing disaccharide, is used as respiratory substrate[9].

The protein content decreased with increasing storage duration. The decrease was more at 15°C than at 10 and 5°C. Since there was a concomitant increase in the protease activity (figure 1), it is evident that proteins were degraded during storage, and the higher the storage temperature, more the degradation[10]. However, there was no significant increase in the total free amino acid content. This suggests that amino acids may be the low molecular weight nitrogenous substances utilized for energy production by the mushrooms during storage, as hypothesized by previous workers[11].

It has been reported that storage duration and higher storage temperatures are correlated with the browning of the mushrooms[3]. The decrease in total phenol content during storage and at higher temperature (table 1) indicates that phenols were utilized for the browning reaction catalysed by the enzyme