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Stomatal studies in Amaryllidaceae with special reference to stomatal abnormalities

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Abstract. Foliar epidermis of nineteen species of Amaryllidaceae, has been studied. Epidermal cells on both the surfaces are polygonal with oblique or straight anticlinal walls in all species except in *Haemanthus* and *Eucharis* where these have sinuous or undulate walls. Subsidiaries are indistinct and are of *C*-type. With the exception of *Eucharis* (hypostomatic) leaves are amphistomatic in all the species. The stomata are anomocytic. Various types of stomatal abnormalities *viz* contiguous stomata, interstomatal cytoplasmic connections etc., are observed in the different species.

Keywords. Amaryllidaceae; foliar epidermis; monocotyledons; stomata.

1. Introduction

The variation and organization of stomatal complex of foliar epidermis of monocotyledons has been given considerable phylogenetic importance (Stebbins and Khush 1961; Tomlinson 1974). Stebbins and Khush (1961) reported anomocytic stomata whereas Shah and Gopal (1970), reported two types—the anomocytic and the paracytic—in a few members of the Amaryllidaceae. This has necessitated an extensive investigation of the stomatal complex so as to assess the types of stomata occurring in the Amaryllidaceae.

2. Materials and methods

The epidermal peels were obtained from young and mature leaves of the following species: *Agapanthus umbellatus* L. Her, *Allium cepa* L, *A. sativa* L, *A. tuberosum* Roxb, *Amaryllis belladonna* L, *A. vittata* Ait, *A. hybrida*, *Nerine curvifolia* Baker, *Crinum zeylanicum* L, *C. album* L, *C. asiaticum* L, *Cyrtanthus mackenii* Hook, *Cooperia pedunculata* Herb, *Zephyranthes candida* Herb, *Z. grandiflora* Lindl, *Haemanthus multiflorus* Martyn, *Eucharis grandiflora* Planch, *Pancratium verecundum* Ait, *Narcissus tazetta* L etc. Epidermal peels were prepared following the technique outlined by Boulos and Beakbane (1971). They were stained with 1 % aqueous safranin solution and mounted in 5 % glycerine. The stomatal index was calculated by Salisbury's (1927) formula.

3. Observations

The cells of the upper (*U*) and the lower (*L*) epidermis are elongated in the direction of the axis. Their anticlinal walls are straight or obliquely oriented except in

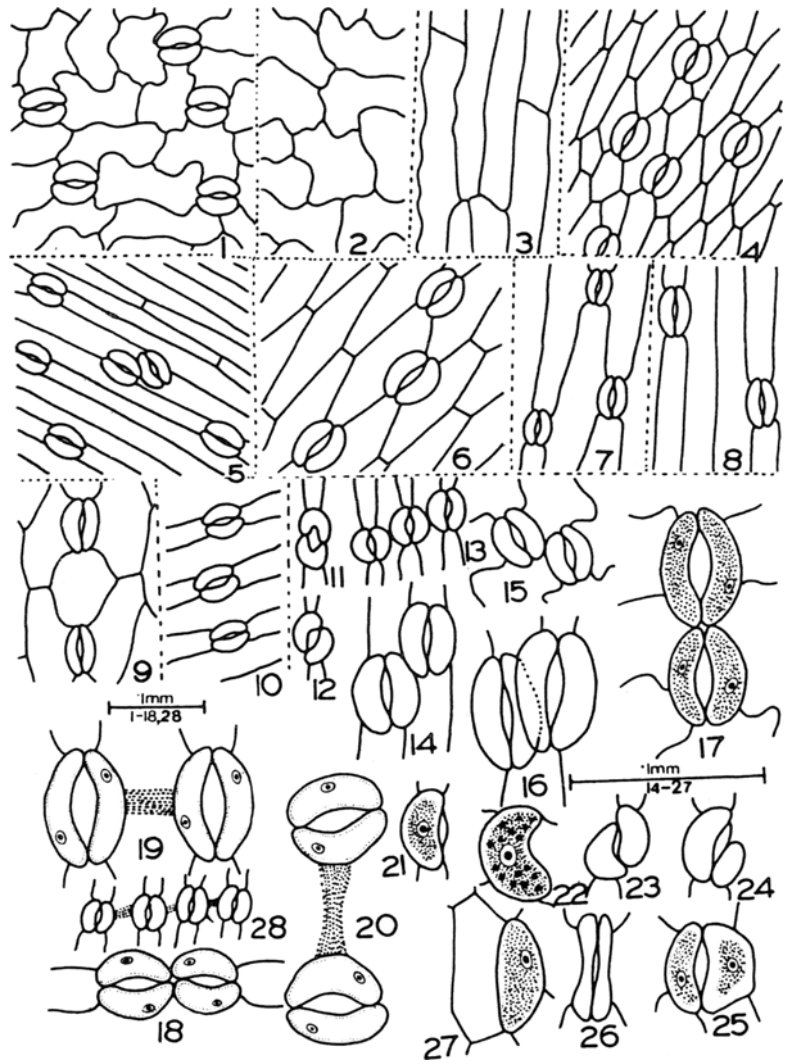
Haemanthus multiflorus and *Eucharis grandiflora* where they are sinuous (figures 1–9) having distinct costal areas. The epidermal cells in the costal area are elongate with undulate walls (figure 3). The frequency of the epidermal cells per mm^2 ranges from $23{\cdot}20 \pm 3{\cdot}15$–$301{\cdot}60 \pm 18$ and $26{\cdot}4 \pm 4{\cdot}29$–$359{\cdot}20 \pm 8{\cdot}59$ on the upper and lower surfaces respectively. The length of the epidermal cells is also variable. It ranges from $164{\cdot}01 \pm 26{\cdot}49$ μm (*Agapanthus umbellatus*) to $304{\cdot}59 \pm 45{\cdot}10$ μm (*Allium sativa*) on the upper surface and $121{\cdot}44 \pm 20{\cdot}50$ μm (*Amaryllis hybrida*) to $301{\cdot}95 \pm 56{\cdot}50$ μm (*Allium cepa*) on the lower surface. On both the surfaces, epidermal cells are covered with a uniformly thick cuticle.

In all the species the stomata are anomocytic (Metcalfe and Chalk 1950). The mature stomata are uniformly distributed in the inter-costal areas and are oriented parallel to the long axis of the epidermal cells. Smith (1935) also suggested a similar type of orientation of stomata in general for monocotyledons. In *A. sativa* (*L*), *A. tuberosum* (*L*), *Amaryllus belladonna* (*L*), *N. curvifolia* (*U*, *L*), *Cyrtanthus mackenii* (*L*) and *Haemanthus multiflorus* (*L*) a few stomata are either oblique or at right angles to the long axis of the epidermal cells (figures 11, 12, 23). The stomata are separated by one to many intervening cells and are surrounded by 3–5 subsidiary cells which are indistinct from the epidermal cells. The leaves are hypostomatic in *Eucharis grandiflora* while in the rest of the species they are amphistomatic. The stomatal frequency varies appreciably in different species but no significant variation is observed in different parts of the same leaf. The stomatal frequency varies from $8{\cdot}80 \pm 3{\cdot}15$ (in *Haemanthus multiflorus*) to $83{\cdot}20 \pm 41{\cdot}43$ (in *Allium sativa*) per mm^2 area on the upper surface and $9{\cdot}60 \pm 2{\cdot}06$ (in *Zephyranthes candida*) to $103{\cdot}20 \pm 13{\cdot}70$ (in *Allium sativa*) per mm^2 area on the lower surface. The stomatal index varies from 7·82 (in *Amaryllis hybrida*) to 70·87 (in *Allium tuberosum*) on the upper and 9·44 (in *Zephyranthes candida*) to 69·35 (in *Allium sativa*) on the lower surface. The size of the stomata ranges between $39{\cdot}29 \pm 1{\cdot}59 \times 28{\cdot}71 \pm 1{\cdot}50$ μm and $57{\cdot}09 \pm 5{\cdot}40 \times 38{\cdot}61 \pm 2{\cdot}22$ μm in different species.

Two types of contiguous stomata were observed (a) laterally contiguous stomata seen in *Agapanthus umbellatus* (*L*), *Allium cepa* (*L*), *A. tuberosum* (*L*), *Amaryllis belladonna* (*U*, *L*), *A. vittata* (*U*, *L*), *Nerine curvifolia* (*L*), *Crinum zeylanicum* (*U*, *L*), *C. asiaticum* (*L*), *Cyrtanthus mackenii* (*L*), *Cooperia pedunculata* (*U*, *L*), *Pancratium verecundum* (*L*), *Narcissus tazetta* (*U*) etc., (figures 13–16) whereas (b) polarly contiguous stomata were found only in *Allium cepa* (*L*), *Allium tuberosum* (*L*), *Amaryllis belladonna* (*L*), *Haemanthus multiflorus* (*L*) and *Narcissus tazetta* (*L*) (figures 17, 18). The laterally contiguous stomata are more frequent than the polar ones on both the leaf surfaces. Polarly contiguous stomata were observed only on the lower surface of the leaf. Sometimes 3–4 laterally contiguous stomata also occur in few species *e.g.* *Nerine curvifolia* (figure 13).

Stomata connected by cytoplasmic strands occur frequently in *Agapanthus umbellatus* (*L*), *Allium cepa* (*L*), *A. sativa* (*L*), *A. tuberosum* (*L*), *Amaryllis belladonna* (*U*), *A. vittata* (*L*), *Nerine curvifolia* (*U*, *L*), *Crinum asiaticum* (*L*), *C. zeylanicum* (*L*), *Cyrtanthus mackenii* (*L*), *Zephyranthes candida* (*U*, *L*), *Cooperia pedunculata* (*U*, *L*), *Haemanthus multiflorus* (*L*), *Pancratium verecundum* (*U*), *Narcissus tazetta* (*U*) etc. (figures 19, 20). Sometimes even up to four stomata are connected by cytoplasmic strands (figure 28).

Stomata with one guard cell smaller than the other were seen in *Nerine curvifolia* (*U*) and *Cyrtanthus mackenii* (*L*) (figure 24). Stomata where one guard cell acts like a subsidiary cell were observed in *Cyrtanthus mackenii* (*L*) and *Pancratium verecundum*



Figures 1–28. Stomata, foliar epidermis and stomatal abnormalities. **1, 2, 3.** Lower and Upper epidermis of *Haemanthus multiflorus* in surface view. **4.** Lower epidermis of *Agapanthus umbellatus*. **5.** Epidermal cells of *Allium cepa*. **6.** Lower epidermis of *Amaryllis vittata*. **7, 8.** Lower and upper epidermis of *Cyrtanthus mackenii*. **9.** Lower epidermis of *Crinum asiaticum*. **10.** Lower epidermis of *Cooperia pedunculata*. **11, 12.** Obliquely oriented stomata of *Nerine curvifolia*. **13–16.** Laterally contiguous stomata of *Amaryllis belladonna*, *Cooperia pedunculata*, *Haemanthus multiflorus*, *Narcissus tazetta*, respectively. **17, 18.** Polarly contiguous stomata in *Haemanthus multiflorus* and *Amaryllis belladonna*. **19, 20.** Cytoplasmic connections between guard cells of two stomata in *Amaryllis vittata* and *Haemanthus multiflorus*. **21.** Stomata with one guard cell and pore in *Cooperia pedunculata*. **22.** Single guard cell without pore in *Haemanthus multiflorus*. **23.** Obliquely oriented stomata. **24.** Stomata with unequal guard cells. **25.** Stomata where one guard cell is like a subsidiary cell in *Cyrtanthus mackenii*. **26.** Degenerated stomata in *Allium cepa*. **27.** Stomata with a single guard cell without pore in *Pancratium verecundum*. **28.** Cytoplasmic connections between four stomata in *Cooperia pedunculata*.

(*L*) (figure 25). Stomata with a single guard cell which occur in *Amaryllis belladonna* (*U*), *Nerine curvifolia* (*U*, *L*), *Crinum album* (*L*), *Cyrtanthus mackenii* (*U*), *Zephyranthes candida* (*U*), *Haemanthus multiflorus* (*L*), *Pancratium verecundum* (*L*) and *Narcissus tazetta* (*U*) (figures 22, 27) are without a pore but others observed in *Allium cepa* (*L*), *Amaryllis belladonna* (*L*), and *Cooperia pedunculata* (*L*) have a pore (figure 21). Stomata with degenerated guard cells were observed in *Allium cepa* (*L*), *Z. candida* (*U*) and *Pancratium verecundum* (*L*) (figure 26).

4. Discussion

In a majority of the species investigated the epidermal cells are polygonal and elongated with oblique or straight anticlinal walls with the exception of *Haemanthus* (tribe-Haemantheae) and *Eucharis* (tribe-Eucharideae). In these instances epidermal cells have sinuous or undulated walls.

In this paper the term subsidiary cell has been used for a cell surrounding the stomata irrespective of the fact whether it is distinct or indistinct. There are different views regarding the presence or the absence of subsidiaries in monocotyledons (Tomlinson 1974). According to Stebbins and Khush (1961), Paliwal (1969) and Tiku *et al* (1978) subsidiary cells are absent in Amaryllidaceae. Usually one indistinct subsidiary cell is common for 2–4 stomata (figures 6, 7, 9). Such type of subsidiaries are classed *C*-type by Ramayya and Rajgopal (1980). According to them such subsidiaries are common on both the surfaces in those taxa in which the costal cells are indistinct. The present observations fully substantiate their views.

In the Amaryllidaceae, Shah and Gopal (1970) recorded both the anomocytic and the paracytic types. But the two genera, *Agave* and *Curculigo*, where paracytic stomata were observed are treated as a separate family—Agavaceae (Hutchinson 1959). Therefore, on the basis of the present observation and those of earlier workers (Stebbins and Khush 1961; Shah and Gopal 1970), it is concluded that the stomata are invariably anomocytic in Amaryllidaceae.

Stebbins and Khush (1961) reported the presence of anomocytic stomata in a majority of Liliales and allied orders, the Iridales, Amaryllidales etc. Pande (1980) also reported anomocytic type of stomata in Iridaceae. According to Hutchinson (1959) there is a close affinity between Liliales, Iridales and Amaryllidales. The present observations support the ideas of Hutchinson. Although Shah and Gopal (1970) reported some sort of connections between guard cells of two stomata in a few members of Amaryllidaceae, they were not sure about their cytoplasmic nature. We find that these connections are actually cytoplasmic extensions of the guard cells which come out through pits in their walls like plasmodesmata. These strands as evidenced are densely stained and granular like the cytoplasm of the guard cells. The cytoplasm of the epidermal cells is lightly stained. These cytoplasmic connections were observed in fifteen taxa including *Amaryllis belladonna*, *Crinum asiaticum* and *Pancratium* sp, where these have already been reported.

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