

STOMATAL ONTOGENY AND PHYLOGENY. I. MONOCOTYLEDONS

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1. INTRODUCTION

The study of the cuticle in living and fossil gymnosperms has made it abundantly clear that stomatal and other epidermal characters are often of great value not only in the delimitation of genera but also in distinguishing the fragmentary fossil remains of allied species (see FLORIN 1931, 1933, 1958). In the early thirties of the present century, two comprehensive works appeared dealing with the systematic value of these characters in the living and fossil angiosperms. The first by ODELL (1932) describes the cuticle in 84 genera of the living angiosperms and the conclusion is reached that none of the epidermal features of the vegetative parts of the living or fossil angiosperms is really satisfactory for diagnostic work. Contrary to this EDWARDS (1935) stated that the structural differences in the leaf epidermis do provide a means of distinguishing some closely related taxonomic groups. He, however, argued that as with other features in classification a sum total of the epidermal characters must be taken into account.

Since the appearance of these two publications, a considerable body of data has accumulated regarding the cuticle. However, in the absence of any comprehensive account, it was thought worthwhile to bring together the available information on this aspect. I propose to complete it under two separate articles, the first one will deal with the monocotyledons and the second with the dicotyledons.

A "stoma" comprises two guard cells surrounding the enclosed pore. In some plants the epidermal cells adjacent to the stoma also become modified in shape, size and contents and are then known as subsidiary or accessory cells. Ontogenetically they may or may not be related to the guard cells. The stoma along with the subsidiary cells, when present, is termed as the "stomatal apparatus" or the "stomatal complex".

As stated earlier, Florin's pioneering work on gymnosperms led to the appreciation of the fundamental value of the epidermal features in interpreting relationships between major groups of plants. The basis for this idea was the opinion held by him that the sequence of divisions leading to the arrangement of cells in the mature stomatal complex is a relatively fixed character in a leaf. Although several recent works tend to suggest that there is much more variation than what Florin had appreciated, his investigations have stimulated a good deal of subsequent work on the morphology and ontogeny of stomata in ferns, gymnosperms and dicotyledons.

As regards the monocotyledons the most comprehensive work is that of STEBBINS & KHUSH (1961) who have attached great phylogenetic significance to

this feature based on two assumptions: (1) that the developmental modes are constant, from organ to organ within a plant, and (2) that the genera and even families exhibit constancy for their possession of a particular complex. A brief survey of the recent literature, however, indicates that in several instances, e.g. *Ananas comosus* (KRAUSS 1948), *Oryza sativa* (RICHHARIA & ROY 1961), *Pandanus* spp. (TOMLINSON 1965), members of the Philydraceae (HAMANN 1966) and still others, the results are at variance with those of Stebbins Khush. The present paper is, therefore, an attempt to evaluate and reconsider some of the prevalent ideas on this subject. The point that deserves main consideration is that these authors appear to have failed to demarcate between observations and inferences and one is unable to understand the bases of comparisons between different families, whether these are purely morphological, or developmental, or both.

2. CLASSIFICATION AND TERMINOLOGY

Although attempts have been made towards classifying the stomata of gymnosperms and dicotyledons (see works of VESQUE, 1889; FLORIN, 1931; METCALFE & CHALK 1950; and later publications of METCALFE; PANT 1965; and GUYOT 1966), no serious thought has been paid to the proper grouping of these organs in monocotyledons. We only know of the gramineous type in which stomata have two lateral subsidiary cells along the dumb-bell-shaped guard cells and are so characteristic of Cyperaceae and Gramineae. More recently METCALFE (1961, 1962) gave the term tetracytic for such stomata which are accompanied by 2 lateral and 2 polar subsidiary cells as seen in some members of the Commelinaceae. However, these two terms are hardly sufficient to demarcate the various types of stomata encountered among the monocotyledons.

In view of the above realization, STEBBINS & KHUSH (1961) made 4 groups of these stomata on the basis of the number, shape and arrangement of the subsidiary cells. In the first group represented in the order Liliales, there are no subsidiary cells. The second category with two or more subsidiary cells surrounding the guard cells is found in members of the Cyperaceae, Gramineae, Haemadoraceae, Juncaceae, Pontederiaceae, and Xanthorrhoeaceae. The remaining two categories have four or more subsidiary cells and are represented in the families Agavaceae, Araceae, Commelinaceae, Palmae, and Pandanaceae.

Although this attempt has helped to remedy the situation to a certain extent, in the absence of any suitable terminology it becomes extremely difficult to imagine any particular type immediately on looking at a given preparation. I would, therefore, like to propose the following classification for the stomata so far recorded among the monocotyledonous plants. Being more familiar with the native ancient language Sanskrit, I have derived these terms from it (p. 664).

1. ASAHKOSHIK¹ – (Aperigenous). These stomata are formed by direct

¹ Sans: 'A' = First letter of Devnagari Script; used to denote without – SAHKOSHIKA = Subsidiary cell. Asahkoshik (adj.) = Without subsidiary cells. DWI = two + sahkoshik; CHATUR = four + sahkoshik; SHAT = Six + sahkoshik; BAHU = Many + sahkoshik.

Table 1. Ontogeny of stomata in Monocotyledons.

Family	Genera and species investigated	Author/s	No. of subsidiary cells	No. of divisions involved
1. Alismataceae	<i>Sagittaria</i> sp.	Stebbins & Khush (1961)	2	2 (+ 1)
2. Amaryllidaceae	<i>Amaryllis vittata</i> ; <i>Narcissus pseudonarcissus</i>	Paliwal (unpublished)	0	1 (+ 1)
	<i>Zephyranthes ajax</i> ; <i>Z. lancasteri</i> ; <i>Z. rosea</i>	Shanks (1965)	0	1 (+ 1)
3. Araceae	<i>Pothos</i> sp. <i>Dieffenbachia sanguinea</i>	Strasburger (1866)	4	4 (+ 1)
4. Bromeliaceae	<i>Ananas comosus</i>	Krauss (1948)	2 or more	variable
5. Butomaceae	<i>Butomus umbellatus</i>	Paliwal (unpublished)	0	1 (+ 1)
6. Cannaceae	<i>Canna edulis</i>	Paliwal (unpublished)	3-7	variable
7. Centrolepidaceae	<i>Centrolepis aristata</i>	Hamann (1963)	2	2 (+ 1)
8. Commelinaceae	<i>Commelina</i> sp., <i>C. communis</i>	Drawert (1941); Stebbins & Jain (1960)	6	6 (+ 1)
	<i>Rhoeo discolor</i>	Stebbins & Jain (1960)	4	4 (+ 1)
	<i>Tradescantia vulgaris</i>	Stebbins & Jain (1960); Campbell (1881); Benecke (1892)	4	4 (+ 1)
	<i>Zebrina pendula</i>	Stebbins & Jain (1960)	6	6 (+ 1)
	Several genera	Porsch (1905); Strasburger (1866); Tomlinson (1966)	2-6	variable
	<i>Arundinaria quadrangularis</i>	Porterfield (1937)	2	2 (+ 1)
	<i>Hordeum vulgare</i>	Shah & Stebbins (1959; 1962)	2	3 (+ 1)
	<i>Oryza sativa</i>	Riccharia & Roy (1961)	0 or 2	variable
9. Gramineae	<i>Phyllostachys pubescens</i>	Porterfield (1937)	2	3 (+ 1)
	<i>Saccharum officinarum</i>	Flint & Moreland (1946); Foard & Haber (1961)	2	3 (+ 1)
	<i>Triticum vulgare</i>	Stebbins & Shah (1960); Haber (1962); Pickett-Heaps & Northcote (1966)	0	1 (+ 1)
	<i>Zea mays</i>	Campbell (1881)	2	3 (+ 1)

10. Iridaceae	<i>Iris</i> <i>Belamcanda chinensis</i>	Strasburger (1866) Paliwal (unpublished)	0 0	1 (+ 1) 1 (+ 1)
11. Juncaceae	<i>Juncus</i>	Stebbins & Khush (1961)	2	3 (+ 1)
12. Liliaceae	<i>Agrostocrinum</i> <i>Allium cepa</i> <i>A. porrum</i> <i>A. sativum</i> <i>Aloe barbadensis</i> <i>Chlorophytum capense</i> <i>Galtonia candicans</i> <i>Hyacinthus</i> <i>Ipheion uniflorum</i> <i>Ornithogallum</i> <i>Sansevieria zeylanica</i>	Paliwal (unpublished) Bünning & Biegert (1953); Stebbins & Jain (1960); Shanks (1965) Paliwal (unpublished) Paliwal (unpublished) Shanks (1965) Strasburger (1866) Shanks (1965) Paliwal (unpublished)	0	1 (+ 1)
13. Marantaceae	<i>Maranta</i>	Strasburger (1866)	0	1 (+ 1)
14. Orchidaceae	<i>Habenaria marginata</i> <i>Orchis</i>	Inamdar (1968) Strasburger (1866)	0 0	1 (+ 1) 1 (+ 1)
15. Pandanaceae	<i>Pandanus graminifolius</i> 4 unnamed species of <i>Pandanus</i> <i>Pandanus fascicularis</i>	Pfitzer (1870) Tomlinson (1965) Pant & Kidwai (1966)	4 4	5 (+ 1) 5 (+ 1)
16. Phylodraceae	<i>Helmholtzia acorifolia</i> <i>H. novoguineensis</i> <i>Orthothylax glaberrimus</i> <i>Philydram lanuginosum</i> <i>Philydrella pygmaea</i>	Hamann (1966)	2-6 4 or more 2 or more 2	variable variable variable 2

division of the meristemoid and are completely devoid of subsidiary cells; e.g. members of the Amaryllidaceae, Araceae, Liliaceae and Orchidaceae. In cotyledonary leaves of *Oryza sativa* a similar situation has been noticed by RICHHARIA & ROY (1961).

2. DWISAHKOSHIK – (Biperigenous). Such stomata are recognized by the presence of 2 subsidiary cells, placed laterally to the guard cells (one on either side). The subsidiary cells owe their origin to the adjacent protodermal cells. Members of the families Cyperaceae and Gramineae are true representatives of this category, with their characteristic dumb-bell-shaped guard cells. They have, however, also been found in Alismataceae, Centrolepidaceae, and Philydraceae.
3. CHATUSHSAHKOSHIK – (Tetraperigenous). Stomata included under this group have 4 subsidiary cells. These may be arranged in two different ways: (a) Two laterals and 2 polars surrounding a stoma as seen in *Rhoeo*; (b) Four laterals, two being located along either guard cell, e.g. in members of the family Zingiberaceae.
4. SHATSAHKOSHIK – (Hexaperigenous). Families such as Commelinaceae, Musaceae and Palmae include members which possess 6 subsidiary cells – 4 being placed laterally and 2 in a polar fashion.
5. BAHUSAHKOSHIK – (Multiperigenous). Such stomata are seen in some members of the Agavaceae, Araceae, Palmae and Philydraceae. These possess more than 6 subsidiary cells which may either be arranged in the form of a ring or irregularly.

As indicated in *table 1* the syndetocheilic (mesogenous) type of ontogeny has not been recorded so far for monocotyledons.

3. DIFFERENTIATION IN THE LEAF EPIDERMIS AND DEVELOPMENT OF STOMATA

Recent studies on the leaf epidermis of several dicotyledonous as well as monocotyledonous families have revealed some interesting points. These are analysed below, in the light of the results obtained during the last 10–15 years.

A. *Differentiation* – It is known that in the net-veined leaves of the dicotyledons the stomata do not differentiate simultaneously but continue to arise through a considerable period of growth of the leaf so that different developmental stages as well as mature stomata occur together. This is in contrast to the parallel-veined leaves of the monocotyledons where the basal regions bear young and developing stomata, whereas those on the older portions have acquired maturity.

According to BÜNNING (1952) the meristematic activity of the young leaf decreases after a period of rapid cell division. However, new meristemoids¹ arise later by resumption of activity at one pole of the protoplasm of certain protodermal cells and these give rise either to a pair of guard cells or a hair

¹ These are the cells which have again acquired some of the characteristics of the meristematic cells.

(also see SINNOTT & BLOCH 1939). The meristemoids are surrounded by a zone of inhibition so that the stomata or hairs develop in the epidermis in a very regular pattern. This arrangement, as Bünning says, is comparable to that of the leaf primordia developing on the shoot apex.

These meristemoids tend to suppress any tendency towards unlimited growth in their vicinity. Thus, until a cell has reached a certain distance from the meristematic region, further division is not possible. Beyond this inhibition zone new meristemoids originate and give rise to regular patterns of stomatal distribution. According to Bünning in several instances, these may cause the neighbouring cells to divide and differentiate into subsidiary cells (*cf.* next title; information available).

Although Bünning's idea of an "inhibition zone" explains the regular arrangement of stomata and hairs, it does not offer any adequate explanation for the frequent occurrence of twin stomata and stomatal triplets in species such as *Millingtonia hortensis*, *Nigella damascena*, *Paeonia anomala*, *Pisum sativum*, *Vicia faba* (unpublished personal observation), *Gnetum ula* (MAHESHWARI & VASIL 1961), *Nicotiana tabacum* (WEHRMEYER 1961) and *Nelumbo nucifera* (GUPTA *et al.* 1968). In fact in *Lonicera japonica* the stomata were found to be arranged in groups of 5 as also in *Pulsatilla albana* where ZIMMERMANN & BACHMANN-SCHWEGLER (1962) recorded 5 or 6 stomata in a row. Contiguous stomata are also induced by the attack of a fungus which may sometimes bring about division of the guard cells (GERTZ 1919a, b). KROPFITSCH (1951) observed as many as 6 stomata placed together in seedlings of *Vicia faba* grown in an atmosphere of ethylene given off by the ripening apples. Hence the occurrence of contiguous stomata both in nature and under the influence of external agents calls for fresh explanation of this peculiar behaviour of the protodermal cells (see also ESAU 1965b¹; PANT 1965)².

In 1866-67 STRASBURGER observed that in members of the family Crassulaceae the subsidiary cells become meristematic and cut off a series of cells. Although YARBROUGH (1934) did not see any division of the subsidiary cells in *Bryophyllum calycinum*, in *Isatis tinctoria* (Cruciferae) and *Basella rubra* (Basellaceae) it has been found that 1 or 2 of the subsidiary cells sometimes divide transversely or longitudinally (PALIWAL 1965a, b; 1969).

It is interesting that a protodermal cell undergoes a series of divisions before becoming the guard-cell-mother-cell whereas the neighbouring cells differentiate either into the epidermal cells or the subsidiary cells. STEBBINS & JAIN (1960) suggested (as also indicated earlier by BÜNNING 1952) that differentiation of the subsidiary cells is due to the influence extended by the guard-cell-mother-cell on the adjoining epidermal cells which are stimulated to divide. The effect of such an induction is either seen on one side or bilaterally (depending whether the

¹ According to Esau (1965b) conclusions of Bünning (1952) do not find support from the experimental works of Hagemann (1957), Reinhardt (1960), and Torrey (1957) on the induction of vascularization in roots.

² Pant (1965) writes "one is, however, unable to explain the simultaneous and gradate sequences of stomata, and sporangial meristemoids on the same basis".

subsidiary cells are formed on one or both the sides) and may be manifested before or after the division of the nucleus of the guard-cell-mother-cell. However, it is not known how this induction brings about the formation of a pair of subsidiary cells on either side of the guard cells. Moreover, in the syndetocheilic development of stomata encountered in the members of the families Acanthaceae, Cruciferae, Labiatae, Magnoliaceae, Theligonaceae and several others, the subsidiary cells are produced one after the other from the same initial. This obviously cannot be explained on the basis of the induction mechanism. In view of this I suggested in an earlier publication (PALIWAL 1967) that in such instances the meristemoid has an inherent capacity to retain its meristematic activity for a longer duration by virtue of which it first produces subsidiary cells and finally becomes the guard-cell-mother-cell.

B. *Development* – During the development of a cell, more or less irreversible changes occur which ultimately result in its specialization. During asymmetrical mitoses, a polarity gradient is set up within the cell causing differences between the two daughter cells at an early stage (BÜNNING & BIEGERT 1953; see also JENSEN 1966). The smaller cell fails to differentiate and remains meristematic.

That there occur considerable nuclear changes in the differentiating leaf epidermis and during the formation of the stomata has been clearly brought out by the study of SHANKS (1965) on *Galtonia candicans* (Liliaceae). He has also compared the formation of the guard-cell-mother-cells (produced by the asymmetrical division of the protodermal cell) with the symmetrical mitoses which take place during the production of the bulliform cells in *Ipheion uniflorum*. He found that the stomatal mother cells divide unequally to produce cells quite unlike in appearance although presumably genetically identical. The larger product, the epidermal cell, has a large nucleolus, and the smaller distal cell (guard-cell-mother-cell) has a small nucleolus. These guard-cell-mother-cells later divide equally to form a simple stoma with 2 guard cells. The latter becomes specialized in form and function.

Nuclear changes occurred throughout the growth period when cell elongation, vacuolation and growth of the wall were taking place. An increase in the nuclear size of all cell types took place, frequently with a change in shape from ovoid or spherical to cubical or pyramidal, and these changes are associated with the increase in DNA, nucleoprotein, and nucleolar volume. Almost without exception, DNA increased to some extent during differentiation. It was noted that as high as 20n ploidy may be found in the epidermal cells of *Galtonia*, where the cell elongation was likewise up to 10 times the original volume. The guard cells grew very little, were more uniform in length and had nearly twice the usual amount of protein and DNA and their nucleolar volume was also doubled when in preparation for mitoses. Where mitoses failed, a more elongated cell resulted, rather like a single, mature guard cell, with diploid to tetraploid values.

Guard cells grew from a length of 16 μ to 40 μ at maturity. They changed in shape, were pulled apart to form a pore, while the wall thickness had increased

and chloroplasts were more numerous. Nuclear size increased from about 6 to 10 μ , and DNA also increased, there being relatively little increase of nucleolar volume. The rate of growth and level of polyploidy reached in these cells, appeared to be associated with the initial supply of nucleolar or cytoplasmic material, or both. It was found that those cells with larger initial supplies of nucleolar material gained nuclear protein and DNA more rapidly, and grew to greater length.

According to Shanks, the rate of development of the cell appears to be associated with its initial supply of nucleolar and cytoplasmic material. Epidermal cells have a larger supply than guard-cell-mother-cells or guard cells. They not only finally reach a much higher polyploidy level, but also develop at a faster rate than their paired partners (epidermal cells).

About the formation of the subsidiary cells Shanks does not seem to agree to the induction phenomenon as suggested by STEBBINS & JAIN (1960) but feels that the development of polyploidy in the epidermal cell at an early stage (before the stoma is formed) may be related to the development of simple stomata, lacking accessory cells.

4. AVAILABLE INFORMATION ON STOMATAL DEVELOPMENT IN MONOCOTYLEDONS

CAMPBELL (1881) investigated the ontogeny of the stomata in *Tradescantia vulgaris*. In mature leaves each stoma consists of two semilunar guard cells surrounded by four (two polar and two lateral) subsidiary cells. Occasionally, five or six subsidiary cells may be found. At the time of initiation of a stoma, a cell undergoes an unequal division. The smaller of the two becomes somewhat elongated. Meanwhile, two lateral and two polar cells are cut off from the adjacent protodermal cells. Next a vertical septum is laid down in the centre of the guard-cell-mother-cell (also the stoma mother cell) dividing it into two guard cells. A pore develops as they mature and the air cavity beneath the stoma enlarges. In *Zea mays* only two subsidiary cells are produced from the adjacent protodermal cells.

PORTERFIELD (1937) studied the development of the epidermis in *Phyllostachys pubescens* and *Arundinaria quadrangularis*. The protoderm of the culms and leaf sheaths is composed of small cells, mostly broader than long, having a large nucleus and dense cytoplasm. Some of these cells function as the stoma-mother-cells. The adjoining protodermal cells cut off lenticular segments which lie next to the guard cell and form the subsidiary cells. The guard-cell-mother-cell itself divides longitudinally to form the guard cells.

The observations of CAMPBELL (1881) and PORTERFIELD (1937) have been confirmed by FLINT & MORELAND (1946) in *Saccharum officinarum*. Thus it is clear that in *Arundinaria*, *Phyllostachys*, *Saccharum*, and *Zea*, the subsidiary cells, although lying parallel to the guard cells, do not arise from the stomatal initial but from the surrounding epidermal cells and that their appearance at maturity may thus be quite misleading (see also ZIEGENSPECK 1944).

Working on *Allium cepa*, BÜNNING & BIEGERT (1953) found that a 3 mm wide zone of dividing cells occurs at the base of the young leaves. Cells of this zone undergo differential divisions to produce (i) large, cytoplasm-poor cells and (ii) small, cytoplasm-rich cells. The latter divide to form the guard cells. Later, STEBBINS & JAIN (1960) also observed that in *Allium* and *Commelina*, in certain protodermal cells the cytoplasm becomes polarized at the distal end and the nucleus then divides by a mitotic figure oriented across the cytoplasmic gradient. Of the two cells the distal divides to form the two guard cells. In *Commelina* divisions also occur in two or more of the surrounding epidermal cells. The divisions are asymmetrical and result in the formation of subsidiary cells in the vicinity of the guard cells. No subsidiary cells are formed in *Allium*.

A similar study of stomatal development was conducted by SHAH & STEBBINS (1959) and STEBBINS & SHAH (1960) in *Hordeum vulgare* and other members of the Gramineae. They mention five main steps: (a) formation of the guard-cell-mother-cell; (b) cutting-off of subsidiary cells by the lateral epidermal cells; (c) appearance of a triad consisting of the guard-cell-mother-cell and subsidiary cells; (d) division of the guard-cell-mother-cell; and (e) completed stomatal complex of four cells.

Some abnormalities in the organization of the four-celled complex include the formation of extra subsidiary cells adjoining the stomatal apparatus; twin stomata; two subsidiary cells on the same side of the guard-cell-mother-cell; and presence of a pair of guard-cell-mother-cell and a short undifferentiated epidermal cell flanked by a large subsidiary cell.

A detailed study of the stomatal ontogeny of 4 unidentified species of *Pandanus* has been made by TOMLINSON (1965). He has confirmed the earlier work of PFITZER (1870) that stomata originate from epidermal cell-files directly above the next innermost hypodermal layer, by the development of substomatal chambers below the future guard-cell-mother-cells. The guard-cell-mother-cells are recognizable by their position immediately above a chamber, but are not otherwise cytologically different from the neighbouring cells of the file. They divide only once by a longitudinal wall which produces the guard cells. Transverse divisions may continue in those cells of the stomatal file which do not function as guard-cell-mother-cells. Such divisions in cells situated at each pole of the guard-cell-mother-cell produce the terminal subsidiary cells. These divisions are never synchronous and may occur early or late, but are usually completed before the division which delimits lateral subsidiary cells. Cells belonging to files on each side of the guard-mother-cell produce lateral subsidiary cells.

Development of stomata does not follow a strict acropetal succession, and stomata at different stages of development occur in a small area of the leaf. In general, however, divisions which produce terminal subsidiary cells are completed first; divisions producing lateral subsidiary cells, which occur throughout in a relatively wide region are completed second; and divisions which produce guard cells are usually last. Divisions within a single complex are rarely synchronous so that only one division figure per stoma is usually seen.

Another point of interest is that the division in the guard-cell-mother-cell is

associated with further internal development. As soon as guard cells are produced, but before the stomatal pore opens, enlargement of the substomatal chamber occurs by separation within the second hypodermal layer. Later, when the stomatal pore opens, there is a communication with the internal atmosphere of the leaf.

According to Tomlinson this type of stoma corresponds to neither of the two main types recognized by FLORIN (1931) in gymnosperms, although in structure it resembles the amphicyclic type. Further, it is almost similar to the development described in *Juncus* and *Sagittaria*, not to that in *Tradescantia* as suggested by STEBBINS & KHUSH (1961).

Table 1 summarizes the available information on this aspect concerning monocotyledons. This clearly brings out that truly syndetocheilic (mesogenous) mode of ontogeny has not been recorded so far for this group of plants.

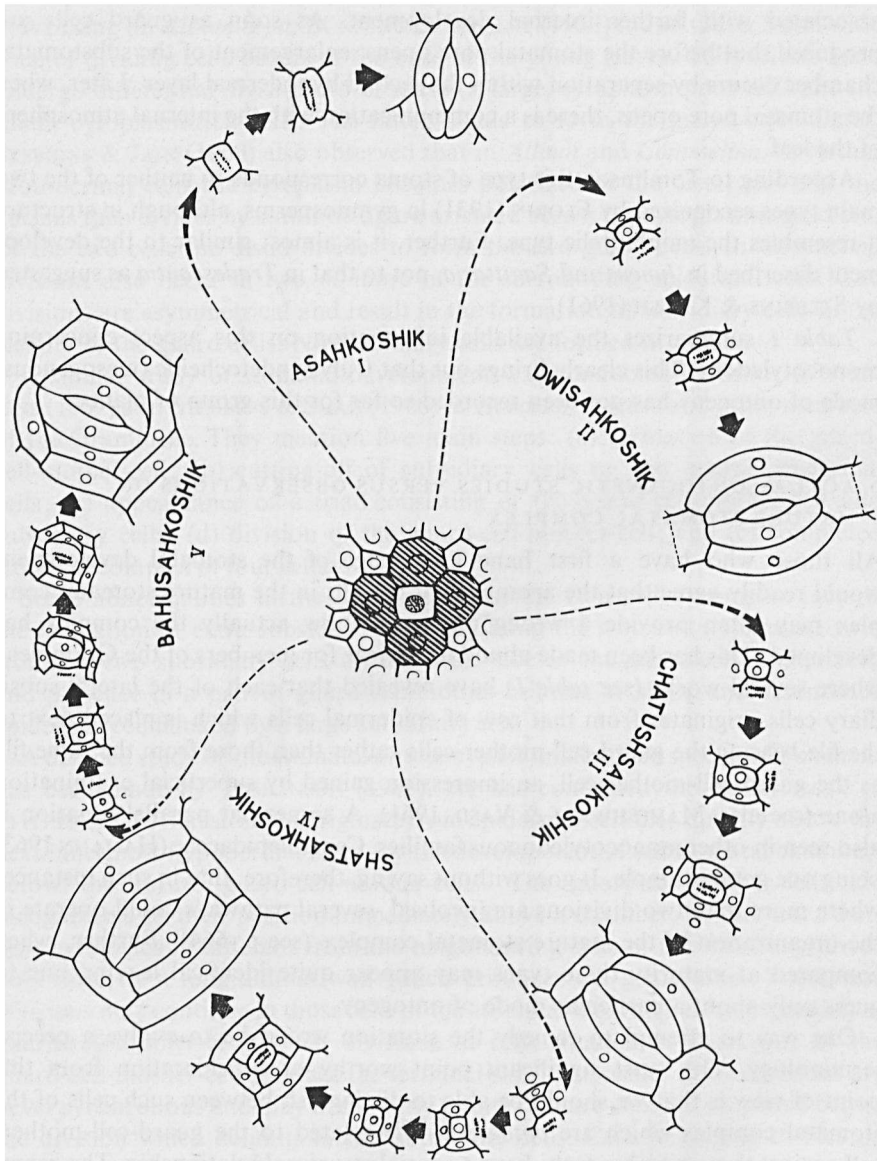
5. ACTUAL ONTOGENETIC STUDIES VERSUS OBSERVATIONS ON MATURE STOMATAL COMPLEX

All those who have a first hand knowledge of the stomatal development would readily agree that the arrangement of cells in the mature stomatal complex may often provide a wrong picture of how actually the complex has developed. This has been made abundantly clear for members of the Gramineae where several works (see *table 1*) have revealed that each of the lateral subsidiary cells originates from that row of epidermal cells which is placed next to the file bearing the guard-cell-mother-cells rather than those from the same file as the guard-cell-mother-cell, an impression gained by superficial examination alone (see also MAHESHWARI & VASIL 1961). A somewhat parallel situation is also seen in other monocotyledonous families, Centrolepidaceae (HAMANN 1963) being one good example. It goes without saying, therefore, that in such instances where more than two divisions are involved, several pathways could operate in the organization of the mature stomatal complex (see p. 664). Further, when compared at maturity these types may appear quite identical leaving one to guess only about a particular mode of ontogeny.

One way to attempt to remedy the situation would be to evolve a precise terminology. The most significant point worthy of consideration from this point of view is that we should be able to distinguish between such cells of the stomatal complex which are *ontogenetically* related to the guard-cell-mother-cell against those which simply have a special *structural* relationship. The general term "subsidiary cell" for both the types of cells seems hardly satisfactory. I suggest that the accessory cells of the first category be designated as SAHODAR SAH KOSHIKA¹ and those of the latter only SAH KOSHIKA². Although PANT's (1965) terminology of Perigenous, Mesogenous and Meso-perigenous offers a very sound basis for comparative investigations on dicotyledons, it cannot be employed for monocotyledons for the simple reason that the leaf

¹ Subsidiary cells borne of the same parents as the guard cells.

² Subsidiary cells originating from adjacent epidermal cells.



development in the two groups is very different. I have, therefore, suggested new terms to be employed in distinguishing the various types in monocotyledons.

It is well established now that the configuration of cells lying adjacent to the guard cells can and do vary considerably. As such it is not always easy to refer to a cell associated with a pair of guard cells, as a subsidiary cell. Examples of this variation in the monocotyledons are provided within a family by Philydra-ceae (HAMANN 1966) and within genera by *Oryza*, *Pandanus* and *Philydrum* (see

also ARRILLAGA-MAFFEI 1966). It seems very probable, therefore, that these different types might have developed in the same way within a taxon. Very often the original pattern is completely lost, after cell division has been completed and elaboration of the complex has taken place, posing a real difficulty in assigning it to a particular type. In fact our knowledge about the development of the stomatal complex in monocotyledons is still too little to allow us to draw any fundamentally sound conclusions.

6. COMMENTS FOR FUTURE WORK

The brief survey that has been presented in the foregoing pages clearly reveals the need of detailed and extensive work on stomatal development in monocotyledons. A survey of the current literature and personal experience of this topic have enabled me to offer a few general remarks for future investigations. These are summarized below:

a. *Effect of polyploidization during ontogeny* – Among others two significant points appear to me to have emerged as a result of SHANKS' study (1965). These are: (a) that there is some correlation between the initial supply of the nuclear and cytoplasmic material and development of a cell, and (ii) that there is a marked difference in the level of ploidy between epidermal cells versus guard cells on the one hand (*Galtonia*) and epidermal cell versus bulliform cells on the other (*Ipheion*). The conclusion, therefore, seems unescapable that the final organization of the stomatal complex is greatly influenced by the degree of polyploidy reached in the various cells of the epidermal tissue.

b. *Influence of underlying layers* – The influence of hypodermal layers on the initiation of the meristemoid and its subsequent development is another aspect which deserves consideration. We have always to bear in mind that the protoderm is the surface layer of an extensive meristem within and not an isolated entity. The only author who has paid attention to this aspect in some detail is PFITZER (1870) who found that in some plants, the position of the meristemoid is determined by an intercellular space in the underlying cells. This subsequently becomes the substomatal chamber (see also CAMPBELL 1881; *Tradescantia*). As TOMLINSON (1965) argues, in *Pandanus* the hypodermal cell files exercise some control on the arrangement of the epidermal files and perhaps the position of the meristemoid itself. For this purpose such leaves which grow by intercalary meristems and possess linear rows of cells provide a suitable source of material.

c. *Sequence of differentiation* – The monocotyledonous leaves which generally have an acropetal sequence of cell divisions and grow by an intercalary meristem provide a convenient material for studies of stomatal development since a continuous developmental series is usually present in one leaf. The findings of DUNN *et al.* (1965) that the length of the guard cells in monocotyledons is relatively more uniform as compared with the dicotyledons can be explained

on this basis without much difficulty. In several families such as Agavaceae, Araceae, Philodraceae and a few others, the guard-cell-mother-cells do not appear to follow a strict sequence of acropetal development. The significance of this variation in comparative studies is an open question.

d. *Formation of the meristemoids and "subsidiary" cells* – As has been indicated earlier monocotyledonous plants are devoid of such cells which may bear any special developmental relation to the meristemoids. The reports of BÜNNING & BIEGERT (1953), STEBBINS & JAIN (1960) and SHANKS (1965) have clearly brought out that in Liliaceae members the meristemoids are formed by unequal division of the elongated protodermal cells. A meristemoid may be easily identified by its smaller size as compared to its sister cell. Suggestions have been made that the guard-cell-mother-cell (meristemoid) may influence the subsequent ability of the associated cells to divide by some kind of "induction mechanism". Although this has been negated earlier (PALIWAL 1967; INAMDAR 1969), suitable explanation is needed for stomatal complexes exhibited by members of the families Agavaceae, Bromeliaceae, Palmae, Pandanaceae, Philodraceae, where a large number of associated divisions take place (*table I*), whereas in members of the Liliaceae there are none. Such developmental differences rather than the "phylogenetic" interpretations of Stebbins Khush would ultimately provide a more useful guideline regarding the distribution of stomatal types in monocotyledonous plants.

Thus careful and detailed investigations on monocotyledonous stomata, not speculations (?) are required to meet the challenge. In this connection the cautious approach advocated by PARKIN in 1924, that in order to draw definite conclusions it is necessary to follow the developmental sequence, since in some cases ordinary epidermal cells parallel to the guard cells may simulate the true subsidiary cells, still holds good and I fully agree with him.

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