IV. DISCUSSION
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The modern biological sciences, like molecular biology and biotechnology, have overpowered the classical and basic biological approaches during the last forty years. In this very strong wave, even the established morphologists and taxonomists have deviated from their fields in the search of the modern knowledge. This is a very serious situation, as we are now facing the non availability of
knowledgeable taxonomists and morphologists. Even for identification of material to be employed for high-tech research, one needs to be helped by the taxonomists. The tissue culturists with the best techniques on hand may yet fail in getting the best outcome if they have no idea of morphogenesis in situ. Professor Vishwambhar Puri, the best known champion of morphology, once and more pleaded for the cause of this neglected branch of botany. When we think of this, stomatal structures come in front line awaiting the answers of a number of unanswered questions. One such question is about the subsidiary cells. As on today, we have no answers regarding their identity, ontogenic status, interrelationship with guard cells, and their function. It was this aspect which was taken up by Patel (1978). Another important aspect related to subsidiary cells is their meaningful involvement in classification of stomata.

In spite of huge information on stomata and their classification has been continuously added to literature, since Pfitzer (1870) published a series of papers on stomatal features, relatively the information is very meagre on subsidiary cells. Morphological and
ontogenetical approaches by most botanists until the recent times relates to the classification of stomatal complex and matters related to it. Some morphologists have employed the techniques of histochemistry for identification of subsidiary cells and their involvement in classification of stomata.

Histochemical studies of epidermal structure were undertaken by Patel et al. (1975) and Murthy and Inamdar (1980), whereas, Pant (1965), Fryns-Claessens and Van Cotthem (1978) and Stevens and Martin (1978a,b) studied the origin of subsidiary cells.

Scanning of literature reveals that very little attention is paid to histochemical studies of stomata and epidermis. Meidner and Mansfield (1968) stated that the histochemistry of stomatal complex is known incompletely, and today, after thirty-two years of their statement, also the situation remains the same. The present work on 123 species describes the qualitative judgement of histochemistry and its relationship with the subsidiary cell and the substomatal chamber.
Subsidiary cells have highly complex histochemical make up, because they are attached physiologically and functionally to guard cells. Ultimately they are responsible for stomatal movement. They also provide a buffer between normal epidermal cell and guard cell.

It is likely that stomata with special type of subsidiary cells may be more active and may participate in plant physiological more activity than those without them. This may also depend on chemical composition, wall characters and cuticular striation on outer walls of subsidiary cells. Moreover, all these aspects are species-specific.

Another important aspect related to subsidiary cells is their involvement in identification of stomata and classifying them to proper position. If one looks at any of the classifications, so far put forth by various workers, it becomes clear that naming the stomata has direct relationship with the maturity status of subsidiary cell/s. Looking into this situation Patel (1979) worked out a new classification of stomata based on various criteria. In fact the very concept of this work (Patel, 1979) has its roots in Patel et.’s work
(1975), wherein they assigned 15 types of stomata in five species of Polypodiaceae. And, out of these two were new types reported for the first time, and four more possible types were suggested for ferns. Thus, it is necessary to identify the subsidiary cells at maturity (Patel, 1978). For this purpose, one has to take their position, shape, size, nature of wall and chemistry into consideration. One or more of these may be met with in a particular case.

I have tried here to develop a comparative picture regarding IP, starch and lipid contents as chemical, and the nature of wall and substomatal chamber as the physical aids.

According to Cutter (1978) epidermal cells of Angiosperms are devoid of well developed plastids except in the submerged leaves of Phyllospadis and Ranunculus.

Hinchman (1973) is of the opinion that the ratio of polysaccharides varies considerably depending on the species or cultivar. The location of PAS positive bodies present in epidermal cells of Cestrum nocturnum and Cestrum diurnum is greater than
that in guard cells. As per the present observations, epidermal cells are normal, subsidiary cells are strong but guard cells are the strongest based on nature of their cell wall and their inclusions.

Strasburger (1866, 67) reported that the subsidiary cells become meristematic and cut off a series of cells. Paliwal (1967) noticed only one or two divisions of the subsidiary cells in *Isatis tinctoria*. It is practically true as the occurrence of a series of cell divisions is not possible at the time of subsidiary cell formation.

Inamdar and Gangadhara (1977) maintained that a subsidiary cell regains meristematic activity and cuts off a small meristemoid which gives rise to either anomocytic or paracytic stomata or arrested development. In *Euphorbia antiquarum* it was noticed that a subsidiary cell of a paracytic stoma became meristematic and cut off a guard mother cell to form anomocytic stoma.

Stomata of various species of Zingiberaceae are considered to have either two lateral subsidiary cells (Fryns-Claessens and Van Cotthem, 1978; Tomlinson, 1969) or two lateral and two terminal
ones (Tomlinson, 1969). However, Stebbins and Khush (1961) considered that many members of Zingiberaceae have 4-5 subsidiary cells arranged around a pair of guard cells in all the four directions. I have reported this in the Commelinaceae, but sometimes, they are arranged at right angle to each other.

Patel (1978) based on the mature structure of stomata considered certain key points to identify the subsidiary cells from other epidermal cells, e.g. they are thin walled, lightly stained and smaller than the other cells. One or more of these characters may be employed to identify the subsidiary cells. These are convenient and easy criteria to identify and distinguish the subsidiary cells from other epidermal cells.

Tomlinson (1969) rightly remarked that subsidiary cells are by definition, structurally modified cells surrounding and often in contact with guard cells. He also made it clear that the cells which bear a developmental relation to the guard cells, but become indistinguishable from other epidermal cells at maturity, are not
subsidiary cells. These cells cannot be distinguished from other epidermal cells, structurally, functionally or physiologically, hence they are not considered to be subsidiary cells.

The subsidiary cells may be ontogenetically related to one or more residual protodermal cells when both the guard cells and subsidiary cells have their origin from the residual protodermal cells. Patel (1978) pleaded for the protodermal origin of all the epidermal components. According to him, the protodermal cell cuts off derivatives which may directly differentiate, or may further divide before differentiating into any of the epidermal components. So, the question of having meristemoidal development of stomata, and, therefore, perigenous or mesogenous origin of subsidiary cells does not find any ground. The ontogenic and mature structures may or may not be similar in all the cases. Tomlinson (1974) has rightly commented, "current confusion arises... for two reasons : first, because the term subsidiary cells is used in two senses both in a morphological and in a developmental context.... and the second source of confusion is the assumption that from the study of the adult
stomatal complex the developmental sequence can be inferred. Inference is still a poor substitute for observation in scientific research."

Esau (1965) defines subsidiary cells as the cells associated functionally with the guard cells and morphologically distinct from other epidermal cells. Tomlinson (1969) clearly states that the subsidiary cells are by definition structurally modified cells. Some of them are related to guard cells in a precise developmental way, whereas others are not. In light of the present analysis, the terminology subsidiary cell, neighbouring cells and contact cells of Tomlinson (1974) are retained. The terms meristemoid, *perigenous*, *mesogeneous* and *mesoperigenous* are not accepted and the term residual protoderm cell (Patel, 1978) is substituted for meristemoid.

Tikku et al. (1978) distinguished subsidiary cells from other epidermal cells using I$_2$KI-H$_2$SO$_4$ test in monocots. With this chemical test epidermal and subsidiary cells react differently. The subsidiary cells show low affinity for PAS and Sudan Black dies, thus
making it distinct from the surrounding epidermal cells. The low affinity for PAS indicates the thin wall of the subsidiary cells. Yet another important aspect to identify the subsidiary cell is the presence of substomatal chamber beneath the entire stomatal complex. This has been clearly demonstrated earlier by Patel (1975, 1978).

A subsidiary cell must be different morphologically and physiologically from other cells. As it is not possible to study the physiological aspects of stomata in mature and dried material, one has to depend on morphology only. But, it must be remembered that the physiological status of subsidiary cell has to be reflected by its morphological and biochemical composition. Therefore, it has been referred here for the identification of subsidiary cells.

The thickening of the wall of the guard cell abutting the stomatal pore is a well known feature, and is related to the opening and closing mechanism of stoma (Meidner and Mansfield, 1968). In Allium sativa the outer wall at the polar region of the stoma shows thickening (Kaufman et al., 1970). In Vaccinium macrocarpon the
walls of the guard cell appear uniform in thickness when seen from the external surface, but when viewed from the side adjacent to the chlorenchyma (sub-hypodermal layer) the ends of the guard cells (common inner wall) appear thick forming pouch-like structures. However, the thickening of the common inner wall of the guard cell in the present investigation is not comparable to such ‘polar vesicles’ or ‘pouches’ as the thickening is observed at all the foci. I am unaware of any previous report of the occurrence of such thickening of the common inner wall or of the outer cell wall at the polar regions of the guard cells except that in *Allium sativa* (Kaufman et al., 1970).