MICROMORPHOLOGY AND
ULTRASTRUCTURE

Although widely used in lower plants, elec-tron microscopy has been a comparatively new approach for flowering plants. The finer details of external features (micromorphol-ogy) have been explored in the recent years by Scanning Electron Microscopy (SEM),whereas the minute details of cell contents (ultrastructure) have been discerned through Transmission Electron Microscopy (TEM). On an average basis, the resolution power of SEM is 250A (20 times asgood as optical microscope, but 20 times lesser than TEM). Behnke and Barthlott (1983) have made extensive studies of SEM and TEM characters. In most of the examples stud-ied, Electron Microscopy (EM) characters proved to be stable and unaffected by envi-ronmental conditions.Micromorphology SEM studies have been made primarily on pollen grains, small seeds, trichomes and surface features of various organs. In most of these organs (except pollen grains), the studies involved the epidermis. The value of epidermal studies lies in the fact that and epidermis covers almost all the organs and is always present, even in herbarium speci-mens. The epidermis is thick and stable in SEM preparations and is little affected byenvironment. However, it is important to note that only comparable epidermis should be studied (e.g. petals of all plants, leaves of all plants, not petals of some and leaves of others). Most of SEM studies have been con-centrated on seed-coats which are usually thick-walled and stable in vacuum, thus fa-cilitating quick preparation for SEM exami-nation without the need for complicated de-hydration techniques. The micromorphology of the epidermis includes the following as-pects:

Primary Sculpture

This refers to the arrangement and shape of cells. The arrangement of cells is spe-cific for several taxa. In Papaveraceae, seed-coat cells by a particular arrangement forma reticulate supercellular pattern (Figure7.6-D), which is a family character. The members of Caryophyllaceae, Portulacaceae and Aizoaceae exhibit a specific arrangement and orientation of smaller and larger cells known as “centrospermoid” pattern.There is specific distribution of long and short cells over the veins in the family160 Plant Systematics Poaceae. The shape of cells is mainly determined by the outline of the cells, boundaries of the walls, relief, and cell wall curvature (flat, convex or concave). Outline of cells may be isodiametric (usually tetrago-nal or hexagonal: Figure 7.6-B and C), elongated in one direction (Figure 7.6-F). Cell boundaries of superficially visible anticlinal walls may be straight (Figure 7.6-B and C),irregularly curved orundulated (S-, U-,omega-, V- types) and are of high taxonomic significance in family Cactaceae and Orchidaceae. Relief of the anticlinal boundary may be channelled or raised. In primitive members of Cactaceae, cell junc-tions are depressed, whereas in derived Cactinae they are raised. The curvature of outer periclinal walls may be flat, concave(Figure 7.6-B) or convex.

Secondary Sculpture

The secondary sculpture (Microrelief) is formed by the deposition of cuticle over the outer wall or due to secondary wall thicken-ings, often shrinking and collapsing in desiccated cells. It may be smooth, striate (Figure 7.6-C), reticulate (Figure 7.6-B) or micro-papillate (verrucose). All members of Urticales have curved trichomes with silicified cuticular striations at the base, and micro-papillations on the trichome body.This single character of trichomes allows for precise circumscription of the order Urticales (Barthlott, 1981). Loasiflorae is circumscribed by unicellular irregularly hooked trichomes. Secondary wall thickenings are always of a high taxonomic significance. In Orchidaceae, for examplelongitudinal striations caused by underlying secondary thickenings are restricted to all members of Catasetinae.

Tertiary Sculpture

Tertiary sculpture is formed by epicuticular secretions such as waxes and other mucilaginous adhesive lipophilic substances and shows a variety of patterns. Secondary and tertiary sculpturing are mutually exclusive as the presence of waxes would invariably mask the cuticle; the cuticle would be visible only if there are no wax deposits. Winteraceae have a particular type and distribution of wax-like secretions (alveolar material not soluble in lipid solvents) on their stomata, similar to gymnosperms, and absent in all other angiosperms.In monocots orientation and pattern of epicuticular waxes seem to provide a new taxonomic character of high systematic significance. Four types of wax patterns and crystalloids have been distinguished (Barthlott and Froelich, 1983):

1. Smooth wax layers in the form of thin films, common in angiosperms.
2. Non-oriented wax crystalloids in the form of rodlets or platelets with no regular pattern. These are common in dicots and Lilianae groups of monocots.
3. Strelitzia wax-type with massive compound wax projections composed of rodlet-like subunits that form massive compound plates around the stomata.

This wax type is found in Zingiberanae,Commelinanae, and Arecanae. It is also found in Velloziales, Bromeliales,and Typhales, which further differ from other Lilianae in a starchy endosperm.

4. Convallaria wax-type with small wax platelets arranged in parallel rows,which cross the stomata at right angle and form a close circle around each polar end of the stoma, similar to the lines of an electromagnetic field. This type is restricted to Lilianae only.

Tertiary sculpture is generally lacking from seeds. In orchidaceae, however, certain tribes possess epicuticular waxes on their seed-coats. Seed-coats of Jacaranda(Figure 7.6-E) have stellate epicuticular sculpture known as ‘star scales’, a feature characteristic of this genus. Many members of Aizoaceae are characterized by seed-coat with epicuticular secretions forming long upright rodlets and small rodlets lying on the cell surface. Cactaceae is a huge family commonly divided into three subfamilies, of which Cactoideae includes 90 per cent species but its classification is difficult because of uniform floral characters, pollen morphology and plasticity. Barthlott and Voit (1979) analyzed 1050 species and 230 genera by SEM forseed coat structure in the family Cactaceae.The simple unspecialized testa of Pereskoideae supports its ancestral position. Opuntioideae has a unique seed with a hard aril, thus confirming its isolated position, also indicated by pollen morphology.Cactoideae shows complex diversity, confirming its advanced position and subtribes have been recognized based on seed-coat structure, each subtribe possessing distinctive features. Thus the genus Astrophytum has been transferred from Notocactinae toCactinae.Orchidaceae is another large family with complicated phylogenetic affinities. Minute ‘dust seeds’ show microstructural diversity of the seed-coat. Studies of over 1000 species (Barthlott, 1981) have helped in better subdivision into subfamilies and tribes.Barthlott also supports the merger of Cypripediaceae with Orchidaceae, a suggestion incorporated in several recent classification (Judd et al., 2002; APG II, 2003;Thorne, 2003; Stevens, 2003).

Ultrastructure

Ultrastructure studies of angiosperms have provided valuable taxonomic information from phloem tissue, mainly sieve tube elements. Besides this, information has also come from studies of seeds.

Sieve-tube plastids

Studies on sieve-tube plastids were first initiated by Behnke (1965) in the family Dioscoreaceae. Since then, nearly all angiosperm families have been investigated
for the taxonomic significance of these plastids. All sieve-element plastids contain starch grains differing in number, size and shape.The protein accumulates in specific plastids in the form of crystalloids and filaments.Thus two types of plastids are distinguished:**P-type** which accumulate proteins and **S-type** which do not accumulate proteins. Starch accumulation is of no primary importance in classification, since it may be present or absent in both types of plastids. P-type plastids are further divided into six subtypes (Behnke and Barthlott, 1983) (Figure 7.7):

(i) PI-subtype.

The plastids contain single crystalloids of different sizes and shapes and/or irregularly arranged filaments. This subtype is thought to be the most primitive in flowering plants, mainly **Magnoliales, Laurales and Aristolochiales.**

(ii) PII-subtype.

This subtype contains several cuneate crystalloids oriented towards the centre of the plastid.All investigated monocots contain this subtype. It is significant to note that only members of dicots with this subtype, Asarum, and Saruma of Aristolochiaceae are widely regarded among the most primitive members of dicots, a possible link between monocots and dicots.

(iii) PIII-subtype.

This subtype contains a ring-shaped bundle of filaments. PIII-subtype is confined to Centrospermae (Caryophyllales) and the removal of Bataceae and Gyrostemonaceae has been supported by the absence of this subtype in these families. Further,forms are recognized based on the presence or absence of crystalloids (Figure 7.8) into PIIIa (globular crystalloid), PIIIb (hexagonal crystalloid)and PIIIc (without crystalloid). Based on the distribution of these forms, Behnke (1976) proposed division of the order into three family-groups whichders Caryophyllineae, Chenopodineaeand Phytolaccineae, earlier established by Friedrich (1956). Whereas Takhtajan had recognized these three suborders in his 1983 revision, in his final revision of his classification, he merged Phytolaccineae with Caryophyllineae, thus recognizing
only two suborders Caryophyllineae and Chenopodineae. Of the three orders recognized in Caryophyllidae of Takhtajan, only Caryophyllales contains PIII-subtype plastids while the other two orders, Polygonales and Plumbaginales, contain S-type plastids. Behnke (1977) as such, ad-
vocated retention of only Caryophyllales under Caryophyllidae and removal of the other two orders to subclass Rosidae whose members also contain S-type plastids. The suggestion was not accepted by Takhtajan(1987) and Cronquist (1988), who retained all the three orders under Caryophyllidae. Takhtajan, however,placed the three orders under separate superorders (Cronquist does not recognize superorders). On further intensive studies of plastids within the group, Behnke (1997) advocated the removal of genus Sarcobatus from the family Chenopodiaceae on thebasis of the presence of PIIIcf plastids and absence of PIIIf, which are characteristic of family Chenopodiaceae. He places the genus in an independent family, Sarcobataceae.

(iv) PIV-subtype.

The plastid contains a few polygonal crystalloids of variable size. This subtype is restricted to the order Fabales.

(v) PV-subtype.

The plastid contains many crystalloids of different sizes and shapes. This subtype is found in the

order Ericales and family Rhizophoraceae.

(vi) PVI-subtype.

The plastid contains a single circular crystalloid. This sub-type is found in family Buxaceae.

Dilated Cisternae

Dilated Cisternae (DC) were first described by Bonnett and Newcomb (1965) as dilated sections of endoplasmic reticulum in the root cells of Raphanus sativus. Originally found in Brassicaceae and Capparaceae, DC have now been found in several other families of angiosperms but are concentrated in the order Capparales (Brassicaceae and Capparaceae) and form a part of the character syndrome of this order. The DC may be utricular, irregular or vacuole-like in form with filamentous, tubular or granular contents. They have been proposed to be functionally associated with glucosinolates and myrosin cells found in this order.

Phloem (p-) proteins

P-proteins are found only in sieve elements of angiosperms and occur in the form of filaments or tubules. These assemble into large discrete bodies, and are not dissolved during maturation of the sieve-element, unlike single membrane organelles. The composition and three-dimensional arrangement of these proteins exhibit taxonomic specificity. They are dispersed over the entire cell as the cell matures but in some dicots, a single non-dispersive (crystalline) body of various shapes may be found in addition to dispersive ones. Crystalline bodies are absent in monocots. Their shape is often specific and thus of taxonomic importance. Globular crystalline bodies are found in Malvales and Urticales. Fabanae, which is characterized by PIV-subtype plastids, has spindle-shaped crystalline bodies in the family Fabaceae. The feature has supported the transfer of Swartzia to Fabaceae.Nuclear inclusions Nuclear inclusions in the form of protein crystals occur in phloem- and Ray parenchyma,primarily in the families of Asteridae. Five types of crystals have been differentiated.Structural differences are significant for classification within Scrophulariaceae and Lamiaceae (Speta, 1979). Protein crystals in sieve-tube elements have also been reported in Boraginaceae, and may prove useful.

Non-phloematic TEM Characters

Protein bodies in seeds through TEM, SEM and dispersive X-ray techniques have demonstrated their significance if qualitative and quantitative aspects are both taken into account. Similarly, SEM studies of starch grains are also potential sources of information of taxonomic significance.

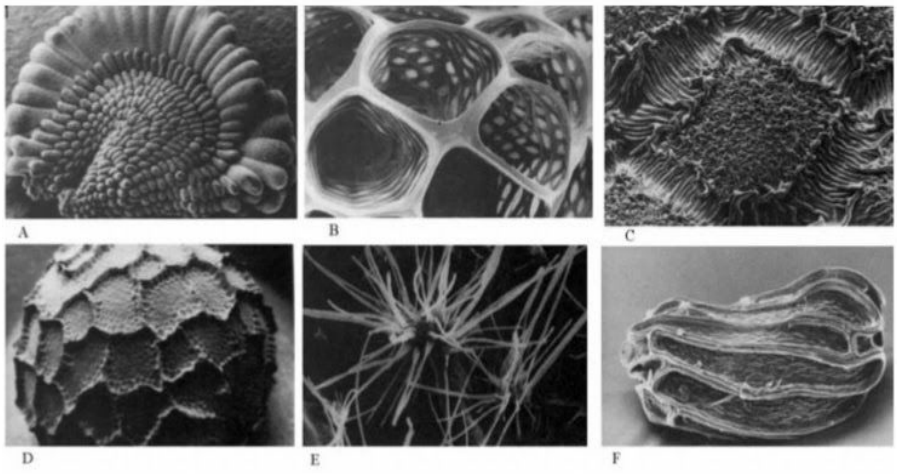

Figure 7.6 SEM seed characteristics of angiosperms. **A:** Seed of *Sceletium campactum* (Aizoaceae) showing centrospermoid cell arrangement; **B:** Seed-coat of *Aeginatia indica* (Orobanchaceae) with isodiametric deeply concave cells and reticulate secondary structure; **C:** Single isodiametric tetragonal cell of seed-coat of *Matucana weberbaueri* (Cactaceae) with heavy secondary sculpturing; **D:** Seed of *Eschscholzia californica* (Papaveraceae) with cells arranged to form a supercellular net-like pattern; **E:** Seed-coat of *Jacaranda macarantha* (Bignoniaceae) with stellate epicuticular sculpture; **F:** Seed of *Dichaea sp.* (Orchidaceae) almost one cell long with heavy marginal thickenings and irregular secondary sculpture. (From Barthlott, 1984).

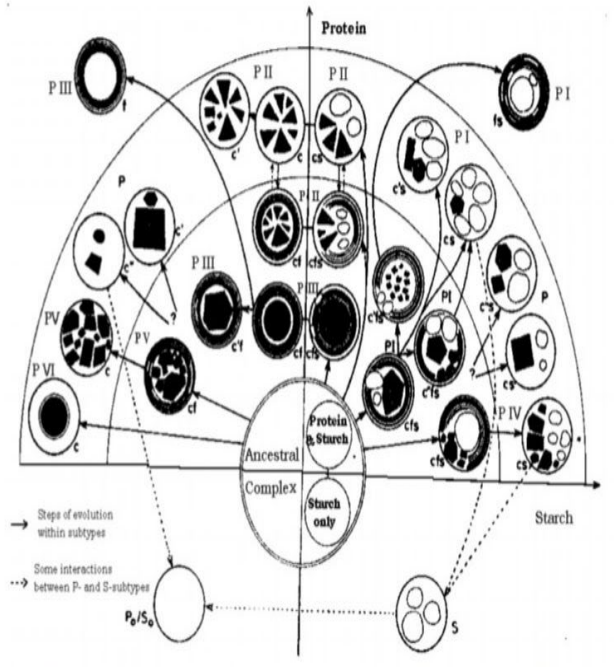

Figure 7.7 Various forms of sieve-tube plastids and their possible evolution (After Behnke and Barthlott, 1983).

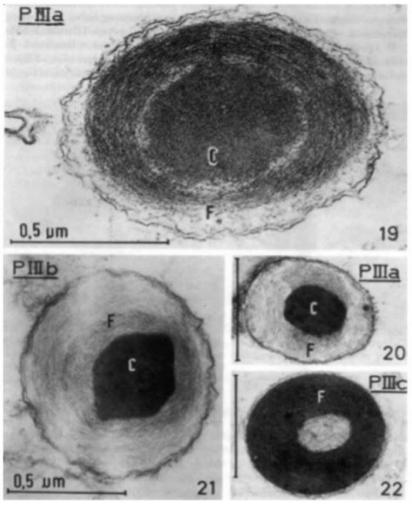

Figure 7.8 Different forms of P III–subtype sieve-element plastids all with ring–like bundle of filaments (F). **19** and **20:** P IIIa with globular crystalloid (C); **21:** P IIIb with polygonal crystalloid; **22:** PIIIc without crystalloid. (From Behnke, 1977).