SUMMARY AND CONCLUSION
Chapter 6
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The pollen grain is the unit of the male gametophyte or their progenitor cells in flowering plants. Each grain contains all the genetic information essential to specify an entire haploid organism. It is empowered to unite with the female gamete at fertilization and form a diploid new sporophyte. The male gametes or the sperm cells formed from the generative cell are housed entirely within the cytoplasm of the vegetative cell.

Pollen grains store carbohydrates in the form of sugars—sucrose rarely glucose or starch. In addition, the pollen contains vitamins particularly of B-group, enzymes, certain hormones as also inhibitors, pigments and traces of inorganic substances such as potassium, magnesium, calcium, copper, iron, silicon, phosphorus, sulphur and chlorine. Several other extractable materials like saturated hydrocarbons, higher alcohols, sterols and fatty acids also occur. The chemical constitution of pollen suggests of plant relationship e.g. rutin is present in the pollen from long styled flowers and quercetin in those from short styled flowers.

To know the chemical constituents and ultrastructure of exine of the pollen grains of ten plants of different families with varied characters have been selected and the following techniques were employed:

- Scanning electron microscopy
- Biochemical estimations using colorimeter
- Histochemical estimations by cytophotometer
- I.R. spectroscopy
- Atomic absorption
spectrophotometry, paper chromatography, electrophoresis and role of pollen diffusates in regulating pH.

The main points in my observations are as follows:

Scanning electron micrographs of pollen grains show that *Aloe* pollen is unicolpate with plano convex, porous exine with zono-reticulate ornamentation. Pollen grains of *Consolea* are spheroidal with rough exine without any spines. Each grain is tricolpate with numerous pores on the exine surface. Pollen grains of *Capparis* are oval with colporate exine usually prolate or subprolate. SEM of *Cassia* shows 3-colporoidate pollen grains having small pores scattered on the exine surface. Pollen grains of *Cycas* are oval with a broad depression of the furrow on the distal side. High magnification shows reticulate type of ornamentation of exine. Oblique pollen grains of *Nymphaea* show a vibrating extent of the operculum. Exine having a broad elliptical or circular operculum occupy the distal part of the grains. Considerably smaller but numerous spines are seen on the exine. The *Polianthes* pollen grains are unicolpate, pseudo operculate, ellipsoidal having thin and wavy colpus margin. It shows reticulate pattern of exine. The pollen is polyrugate, monocolpate and spheroidal, spinules about 2.2 μm are seen on the exine surface. The colpae is like a longitudinal aperture. *Turnera* pollen is subprolate, trizonocolpate, having triangular shape provided with projection towards the angle. High magnification shows reticulate ornamentation.
The pollen grain of *Najas* is ellipsoidal but rounded provided with an intine and a fringe of the exine. The SEM study and cross section of pollen as observed under light microscope reveal an extremely thin exine.

The pollen grains have two walls: exine and intine. The exine is composed of extremely resistant substance the sporopollenin. In the arms of the exine of all pollen grains, perceptive or guiding proteins were not observed. It is likely that high temperature and mounting removed all proteins.

Biochemical estimations revealed that *Jatropha* pollen contained a minimum amount of proteins (4.94 µg/mg) but maximum amount of DNA (27.56 µg/mg). In pollen grains of *Aloe* proteins (59.68 µg/mg), reducing sugars (181.54 µg/mg) and total sugars (201.14 µg/mg) are highest. In *Turnera* pollen RNA (59.04 µg/mg), amino acids (137.76 µg/mg) and non-reducing sugars (31.36 µg/mg) are at its peak level. Amount of soluble proteins (14.80 µg/mg) and phenols (58.50 µg/mg) are higher in the pollen grains of *Cassia* (fertile anthers) in comparison with sterile anthers (11.45, 55.04 µg/mg respectively). In *Cassia* fertile, partly fertile and sterile anthers the amount of proteins increases while that of aminoacids decreases. All pollen grains contain phenols. Pollen grains of *Cassia* (fertile) contained a very high amount of phenols (58.5 µg/mg) while they were extremely less in the pollen of *Cycas* (6.7 µg/mg).
Histochemical estimations revealed that the highest amount of proteins (5 x 10^{-3} \text{ AU}) and RNA (1.73 x 10^{-3} \text{ AU}) per unit area in the pollen grains of Capparis while they were minimum in Consolea (1.34 x 10^{-4} \text{ AU}) and Polianthes (1.64 x 10^{-4} \text{ AU}) respectively. In Turnera pollen, amount of DNA per unit area is minimum (4.03 x 10^{-5} \text{ AU}) while it is maximum in the pollen of Cassia (partly fertile anthers) 8.77 x 10^{-4} \text{ AU}. In Aloe pollen the amount of insoluble polysaccharides per unit area is extremely less (2.22 x 10^{-5} \text{ AU}). In Polianthes concentrations of RNA (1.64 x 10^{-4} \text{ AU}) per unit area of pollen is minimum. Ascorbic acid was found extremely less in the pollen grains of Portulaca (3.9 x 10^{-4} \text{ AU}) while it was maximum in the Capparis pollen (6.4 x 10^{-3} \text{ AU}).

A stupendous increase of endogenous ascorbic acid content during gametogenesis is followed by a simultaneous increase in RNA content per cell. This rise is associated with the external morphological changes of double fertilization. It heralds a derepressive action of ascorbic acid. To open or free the repressor effect, the chemical qualifies for the following: It should decrease the histone or acidic protein content within the framework of the nucleus. It should increase m-RNA synthesis. Ascorbic acid satisfies both the above properties of a derepressor molecule.

The escaping pollen has two nuclei. The vegetative nucleus is arginine rich while the generative cell or its two male gametes contain lysine rich fraction of histones.
These qualitative estimates imprint functional diversity.

IR (KBr) of the pollen grains of all the ten plants manifested frequencies mainly for broad-OH stretching in the region around 3400 cm\(^{-1}\), -CH stretching vibration near 3000 cm\(^{-1}\), and a weak to medium ketonic vibration around 1750 cm\(^{-1}\) and 1650 cm\(^{-1}\). The ketonic vibration has to be strong one but due to hydrogen bonding weak to medium ketonic vibration is observed. Thus two functional groups are present (i) \(\text{C} = \text{O}\) group and (ii) -OH group. A carboxylic acid is present in pollen grains of all plants analysed. Moreover, the IR spectra suggested the same functional group present in all the pollen under study but they differ in their composition.

By IR spectra of extract of rubber plant, -SH group (2650 cm\(^{-1}\)) is confirmed only in Mexican guayule rubber. The -OH group (3400 cm\(^{-1}\)) is confirmed only in guayule resin. Ketonic vibration (1700 cm\(^{-1}\)) was not observed in Hevea rubber which is located in all the remaining three rubber samples. These results point to the structural difference between four varieties of rubber viz. Mexican guayule rubber, guayule resin, guayule rubber and Hevea rubber.

Inorganic elements detection in the pollen grains by atomic absorption spectrophotometric analysis revealed the presence of extremely less amount of copper in sterile anthers of Cassia (7.65 \(\mu g/g\)) compared to partly fertile (9.18 \(\mu g/g\)) and fertile anthers (13.84 \(\mu g/g\)), K\(^{+}\), Ca\(^{2+}\), Mg\(^{2+}\) and Na\(^{+}\) were found as important nutrients. Cu\(^{2+}\), Zn\(^{2+}\) and
Mg$^{2+}$ were highest in *Jatropha*. Na$^+$ was highest (8000 μg/g) in the pollen of *Nymphaea*. Fe$^{3+}$ was also highest in *Nymphaea* pollen. Pollen grains of *Aloe* contained the highest amount of Ca$^{2+}$ (3907.8 μg/g) which was not detected in *Nymphaea* pollen. *Turnera* contained highest K$^+$ (44,800 μg/g).

Heavy metal ions such as Cd$^{2+}$, Cr$^{3+}$, Pb$^{2+}$ were not detected in sterile anthers of *Cassia*. Essential nutrients like Na$^+$ and Fe$^{3+}$ were also not detected in *Cassia* sterile anthers.

Chromatographic separation of amino acids suggested the presence of three unique amino acids (serine, isoleucine and leucine) in *Consolea* pollen, proline was detected in all the ten plants, phenyl alanine in eight plants, glycine in seven, methionine in six, valine in six, aspargine in five, tyrosine in four, glutamic acid in three, aspartic acid in two, cystine in two, a- alanine in two and threonine in two plants. Thus amino acids, differ from a pollen of one plant to another.

Chromatographic separation of carbohydrates revealed that the pollen grains of *Aloe*, *Cycas*, *Jatropha*, *Polianthes* and *Turnera* contain glucose and fructose. The pollen grains of *Cassia* contain only fructose and pollen grains of *Nymphaea* contain only glucose. Sucrose was confirmed in *Consolea*, and *Portulaca* while raffinose was confirmed only in *Capparis* pollen.

The above wide dispersal leading to the only act of fertilization carve out a different chemical pathway for the pollen and equip them for resistance to wind, water and
insect transport.

The number of proteins and their molecular weight in different stages of pollen development in Consolea was confirmed using SDS-PAGE method. Proteins varying from 14,200 to 5,45,000 molecular weight were located in all stages. The number of proteins varied from 8 to 14. Maximum proteins bands were observed during immediate anthesis. Increase in protein bands from second to fifth stage of pollen development and its decrease in the sixth stage (post anthesis) is remarkable. Three to four proteins are located uniformly in all stages of development between the mole- weight of 14,200 and 29,000. Three proteins were located between the mole. wt. of 66,000 to 5,45,000 upto anthesis stage. No proteins of mole. wt. 66,000 to 5,45,000 were located in the sixth stage (post anthesis) of pollen development.

Protein pattern did not show any difference during the early stages of development. Changes in the protein profile were frequent during the last stages. (Pre, post and at the time of anthesis). There was a decrease in protein number at fifth stage, it was 12 in preanthesis stage (4th stage). Increasing in protein bands was observed in sixth stage (fifteen bands). All the fifteen bands were located between the molecular weight of 14,200 to 66,000. There were no proteins at high mole. wt. (about 66,000).

Effect of pH on the germination medium, release of amino acids and proteins from the pollen wall and their role
in pH regulation was studied in ten plants. The germinating pollen releases amino acids, proteins and a range of other chemical moieties into the culture medium.

Pollen diffusates alter the pH of the external germination medium within first ten minutes of incubation and does not affect the pH after 20 minutes of incubation, suggests that substances responsible for pH shift diffuse within the first 10 min. Thus prolonged incubation does not significantly change the pH.

The pH of the media having initial pH 3 to 6, (except the Cassia and Polianthes pH 3 to 7, and in Cycas at pH 3 to 4) increased and became less acidic while media with pH values of 7 to 10 decreased and becoming less basic. Thus the pH change is not controlled by proton fluxes alone but involve a release of buffering compounds. This indicates that diffusates have acidic and basic substances and have a strong buffering capacity. The maximum release of proteins and amino acids in media, does not correspond with the pH of medium in which maximum pH shift is observed. This provides a further evidence that the proteins and amino acids released into the pollen germination medium do not have a direct involvement in pH regulation but inorganic ions and organic acid anions are also be involved.

Each pollen grain is fully equipped with the paraphernalia of an independent plant. It has led to successful raising of haploids in tissue culture. The development is well determined and unidirectional. There are
neither fluctuations nor upheavals. Inspite of the seemingly identical chemical constitution with different concentrations in picograms and inspite of the identical chemical pathways employing different concentrations, the developmental routes of pollen are convergent. The biochemicals equip the pollen for effective double fertilization. The preparation remains unidirectional.