DISCUSSION
Chapter 5
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The process of meiosis leading to the constituents of pollen grain for double fertilization marks a significant event in the reproductive cycle of a flowering plant. Four aspects of this process shape the whole compendium: (1) regulatory mechanism (2) energetics (3) hereditary complements (4) external environment. First three aspects of differentiation are discussed here with reference to chemical make up of pollen grain. The problem still hankers round whether these isolated fragments would yield to a total or a whole understanding of the individual.

A study of the process of pollen development and its chemical alignment is one of the most charming and exciting fields of developmental biology. During its ephemeral life span, chemical signals travel rapidly to develop in one direction rather than in another. If the totipotency of the pollen is conceded, the question arises as to why development takes place unidirectionally and not as a wild uncontrolled growth which would result if the entire geno-chemical apparatus were to be functional all the time. The present study attempts to evaluate the correlation and co-existence of ascorbic acid with nucleic acids, histones, three species of proteins, carbohydrates, DNA, RNA, free amino acids, phenols, during the course of pollen genesis.

Pollen morphology:

Sporopollenin leaves various patterns of sculptures e.g. zono reticulate ornamentation of exine in Aloe, reticulate in Cycas, Polianthes and Turnera and pilate in Jatropha and
resides in its crevices, the guiding proteins detected by scanning electron microscope. In its make up it keeps areas without the deposition and forms colpi (e.g. pollen grains of Aloe and Polianthes are monocolpate, those of Consolea and Turnera are tricolpate while Jatropha pollen is non colpate), pores (e.g. Aloe, Consolea, Cassia), furrows as in Cycas) and apertures. However Jatropha pollen is non aperturate. Nymphaea pollen has an operculum occupying the distal part of the grain.

Histones:

Cytochemical detection of basic proteins (histones) with three different chemicals reveals a differential staining reaction in pollen nuclei. Vegetative nucleus contains intense arginine rich histones as evident from brownish black staining with ammoniacal silver nitrate. On the contrary the generative nucleus shows yellow staining reaction characteristic of lysine rich histones.

The vegetative nucleus contains several times more arginine rich histones than that of its counterpart generative nucleus points to its active stage. Sauter reported a very high content of DNA associated histone which was lysine-rich in the generative nucleus and no lysine rich histone in the vegetative nucleus. Further lysine rich histones cross-linking the chromatin fibrils are responsible for the formation of dense inactive chromation as compared to arginine rich ones. Thus no RNA is observed in the generative cell as the dense chromatin being inactive with respect to
DNA-directed RNA synthesis.  

Proteins and amino acids:  

The free amino acids were identified by chromatography by comparing with the $R_f$ values of the authentic samples. Bound amino acids were identified by extracting proteins and then hydrolysing by an acid. Arginine and proline dominate in the pollen grain.  

The stigma-pollen interface acts as a rehydration point and the insulated pollen wall gives way. Osmotic balance between pollen and stigma papillae induces water flow from stigma to pollen and thus unfolds its germination.  

$pH$:  

Although pollen in germination media at $pH$ 8.0 or higher, lowers the $pH$, the increase in $pH$ in acidic and neutral media towards a value of $pH$ 7.4 - 8.0 point that the $pH$ change is not controlled by proton fluxes alone but involves a release of buffering compounds. The pollen grain of *Malus* 56 and *Crotalaria* 60 also modifies the $pH$ to 6.0 to 6.25 respectively. The release of leachates from *Ipomoea* 181 does not alter the $pH$ further after 30 minutes of incubation. Thus chemicals responsible for $pH$ shift released within first 25 minutes and the release of substances from pollen tubes after germination does not significantly change the $pH$. It indicates that the substances responsible for $pH$ change within the first ten minutes of incubation originate from the pollen wall.
It also points that the rate of release of amino acids and proteins are pH dependent. The amount of the material lost is a function of pH of eluting medium. The relation between pH of the medium and release of protein and amino acids is still in oblivion.\textsuperscript{182} The reverse is unlikely and does not seem to be the case, since the maximum release of proteins at pH 7.0 and amino acids at pH 8.0 does not correspond with maximum pH shift at pH 4.0.

Two major conclusions can be drawn from the results. First of all these species show maintenance and regulation of their internal pH levels. Second, there are differences both in the actual values of the internal pH levels maintained and in the response of internal pH to external pH dependent upon whether a species is acid tolerant or acid intolerant.

Sterility:

The chief aim of this analysis is (1) to detect chemical differences of AA, RNA, histones, phenols between fertile and sterile stamens of the same flower and those of the male and female flowers.

Ascorbic acid/-SH proteins and sterility:

Stern and Timonen\textsuperscript{183} showed that the twin occurrence of ascorbic acid and -SH groups in pollen of Lilium is responsible for respiration as these two substances act as hydrogen carrier in biological respiration. The low concentration of one of them, in this case ascorbic acid is associated with sterility. But what happens to pollenocytes if one of the two is absent remains a problem still.
Nakashima and Hishikawa\textsuperscript{10} proved that pollenocytes of sterile anthers of maize inherit sterility via its cytoplasm. However the translocation of the photosynthates like sugar and its derivatives into the anther is obstructed in male sterile lines. Surprisingly the movement of ascorbic acid is obliterated in sterile anthers of the female male flower and subsequently contributes to sterility.

According to recent studies there may be other factors, apart from nutritional disharmony responsible for pollen abortion. These studies indicate the irregularities in the deposition of callose around the microspore mother cells and its timely breakdown are responsible for male sterility.

Male sterility at lower concentration of the chemical is largely due to the failure of anthers containing normal pollen to dehisce. At increasingly higher concentration, sterility was due more to the failure of pollen development.

The pair of sperms in the pollen grain does not show any detectable differences. But they have to fulfil quite different roles during double fertilization. Russel and Cass\textsuperscript{148} suggest that both the male gametes have quite different patterns of male cytoplasmic transmission. The tricellular pollen (\textit{Cycas}, \textit{Jatropha}, \textit{Portulaca}) always responds to rapid germination and pollen tube growth. While water dispersed pollen of \textit{Naias} has a thick intine and exine fragile or is totally absent. \textit{Naias} snake venom did not respond to germination as observed by Singh and Malik.\textsuperscript{185}
Electrophoresis:

Method of characterizing proteins by disc gel acrylamide electrophoresis was applied to pollen grains by Linskens. It recorded 15 to 25 recognisable protein bands. Differences were also noted in the densitographs of the respective protein electrophorograms.

The pollen is surrounded by a sporophytic layer of nurse cells, the tapetum. Pollen mother cells undergo meiosis to form haploid pollen grains that reach maturity in buds one day prior to anthesis and mature pollen grains are shed as the anthers dehisce in the open flower. Pollen maturation is accompanied by changes in the sporophytic tissues of the anther. In particular, the cells of the tapetum become disorganized and dissolve. Electron microscopic studies indicate that material from the tapetum is transferred into the outer layer of the pollen wall, the sculptured exine.

The viable pollen grains release an exudate which becomes continuous with the stigma surface and is accompanied by an enhancement of surface esterase activity. The attachment of pollen during the contact face is enhanced by a rapid increase of the esterase activity.

IR Spectroscopy:

In IR spectroscopy, 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 is a strong one but due to
hydrogen bonding weak to medium ketonic vibration is observed.

All IR spectra converge in the same direction for pollen of different genera and species and families and even habit and habitats. It is uniform for the all pollen grains.

Rubber:

The modified extraction method withstood the rubber and resin IR testing and showed peak values at 2650 cm\(^{-1}\), in Mexican guayule rubber confirmed -SH group, 3400 cm\(^{-1}\), in guayule resin confirmed -OH group, 1750 - 1650 cm\(^{-1}\) confirmed ketonic group in Mexican guayule rubber and resin. The intense detection of rubber cells below the inflorescence head is quite unique as the rubber is inert. How can it harbour a flowering hormone?

Callose:

Differential behaviour of callose deposition is a charming field of study. However its ephemeral appearance in the pollen tubes heralds its control of gamete transport. The callose disturbance induces male sterility also.  

External Ca\(^{2+}\) is essential for callose induction and inhibition of Ca\(^{2+}\) uptake by putative Ca\(^{2+}\)-channel blockers decreased callose synthesis. Increasing Ca\(^{2+}\) uptake alone appears insufficient for the induction of callose formation. Some of the substances capable of rapidly inducing callose synthesis has also been shown to elicit the slower production of photo alexins suggesting that the signal transduction mechanism involved in callose synthesis
contribute to the regulation of other unidirectional metabolic pathways.