Quadrant II – Transcript and Related Materials

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Notes

Microgametophyte (Development of Male Gametophyte)

The male gametophytic generation begins with the formation of microspores. The microspores are released in the anther locule by the breakdown of the common callose wall of the spore tetrad. However, the microspores start synthesising their individual callose walls to their release. The microspores after release from the tetrads are known as pollen grains and represent the first cell of the gametophyte

Microgametogenesis

Microgametogenesis involves several dividing steps, starting from pollen germination and culminating in the formation of male gametes. Pollen germination starts while it is within the pollen sac. After being released from the spore tetrad, grain absorbs locular fluid and expands considerably. Vacuoles make their appearance and the cytoplasm gets limited to a thin film lining the cell wall. The nucleus shifts from the centre to the periphery and prepares to divide mitotically. Nuclear division is asymmetric and gives rise to two unequal cells, the larger vegetative cell and the smaller generative cell.

However, before the mitotic division starts in a pollen, two important changes are observed-

(i) Migration of nucleus—The nucleus shifts from the central to a peripheral position. which is in a definite direction and marks the position of the generative cell. This position is always constant and is genetically controlled in each species. However, some variations have been found, as in *Spiranthes*, the generative cell is cut off towards the distal pole (opposite to the tetrad centre), and in *Drimys*, it is cut off on the proximal pole (towards the centre of the tetrad).

(*ii) Polarisation of cell organelles*—The cytoplasm becomes highly vacuolated, resulting in the displacement of the cell organelles. Polarisation of the cell organelles occur and most of the plastids, mitochondria and lipid bodies migrate towards the region which is to form the vegetative cell.

Formation of vegetative & generative cells

Nuclear division is followed by cytokinesis resulting in the formation of a large vegetative cell and a small spindle-shaped generative cell. The partition wall between the two cells finally disappears and the generative nucleus lies within the cytoplasm of the large vegetative cell.

Vegetative cell: The vegetative cell is large and naked occupies a major part of the pollen grain. After divisions of the pollen nucleus, the vegetative nucleus continues to grow. The cell organelles like plastids and mitochondria increase in size as well as in number, The RNA and protein contents increase considerably followed by a decrease in a vacuole size, which soon disappears. In several plants the vegetative cell contains stacked, rough ER which are released during pollen germination. Rough ER probably contains storage products, necessary for pollen germination and growth of the tube. The storage products are usually reserve RNA and proteins, as these are required during pollen germination. The nucleus also shows considerable changes. The nuclear membrane becomes highly convoluted and the nucleolus becomes prominent. However, in mature stages the nucleolus disappears.

Generative cell. In the early stage of pollen division, the vegetative and the generative cell are separated by two plasma membranes. Later. wall formation starts between the cell membrane of the generative cell and the intine of the wall until the two ends of the growing wall meet and fuse and the generative cell is pinched off.

The nature of the wall of the generative cell has been variously interpreted. Some workers report the presence of a definite cell wall, others state that only an electron transport layer is present between the two cell membranes, whereas others suggest that there is no wall around the generative cell. However, the absence of true cellulosic cell wall is confirmed. Echlin (1972) reported the presence of callose wall in majority of plants, while Owen and Westmuckett (1983) reported the presence of B-l, 4-glucans along with callose deposition.

The generative cell is small in size and spherical in shape at the time of detachment from the pollen wall. During pollen development, it changes shape, becomes elongated and appears vermiform. The elongated shape of the generative cell facilitates its movement into the pollen tube.

The cytoplasm of the generative cell is hyaline, highly reduced and contains the cell organelles like mitochondria, ribosomes, ER, microtubules and dictyosomes. Initially when the generative cell is spherical in shape, the microtubules are scattered randomly in the cytoplasm, but as the cell assumes elongated shape, the microtubules become oriented with their long axes parallel to the long axis of the cell. Hence, these axial microtubules play an important role in maintaining the elongated shape of the generative cell. Eventually, the generative cell loses contact with the pollen wall and shifts into the vegetative cell. The pollen grain, thus, becomes two-celled.

The function of the generative cell is to give rise to the male cells, i.e. the male gametes or sperms. The generative nucleus divides by mitosis followed by cytokinesis, and a wall separates the two cells. The partition wall, as well as the surrounding wall of the sperm disappear and the naked sperms are released- An important feature of the sperm formation is that their separation is caused by degradation of the partition wall and not by the furrowing of the binucleate cell. The cytoplasm of male cell generally contains the organelles present in the generative cell.

Nuclear division in the generative cell may take place under two conditions-

(i) while the pollen grains are confined within the anther, i.e. pollens are shed at the three-celled stage, as in *Beta* and *Hordeum*, or

(ii) after the pollen has been shed from the anther, i.e. at the two-celled stage.

In the two celled pollen grain, the generative cell may divide, after the pollen has alighted on the stigma (e.g. *Holoptelea integrifolia*) or in the pollen tube just before it reaches the embryo sac. The latter case is the most common method. In rare cases, the generative cell divides after the pollen tube has reached the embryo sac (e.g. *Euphorbia terracina*).

However, in majority of plants, the generative nucleus divides before the pollen has shed, i.e. at the three celled stage. The division of the generative nucleus at an early stage is an indication of the suppression of the male gametophyte-a characteristic feature of vascular plants.

<u>Differences in the behaviour of vegetative and generative cells</u>. The two cells, vegetative and generative, though arising from a single parent cell exhibit different behavioural patterns, which are based on nuclear and cytoplasmic differences between them. Differences are as follows:

Generative cell	Vegetative cell
After pollen mitosis, DNA content increases 2C level	After pollen mitosis, DNA content increases to 1C level
Lysine rich histone is associated to DNA.	It is absent.
RNA and protein synthesis practically absent.	RNA and protein synthesis are excessively high
The generative cell is metabolically inactive.	The vegetative cell is metabolically active and supplies the required metabolites to the generative nucleus through cytoplasmic connections.

<u>Internal organisation of mature pollen grains</u>

Brewbaker (1957) reported that about seventy per cent of the angiosperms (e.g. Solanaceae and Rosaceae) possess bicellular pollen grains whereas thirty per cent (e.g. Cruciferae, Compositae, Caryophyllaceae and Graminae) exhibit tri-cellular pollen grains. Both types of pollen grains have a single vegetative cell and a single generative cell. The generative cell later produces two male gametes. The two male (sperm) cells lie in the cytoplasm of the vegetative cell enclosed by their individual cell membranes as well as an internal membrane of the vegetative cell.

The work done on three-celled pollen grains of Brassica spp., *Spinacea oleracea* and *Zea mays* reveals that the sperms exhibit remarkable dimorphism. In

Plumbago zeylanica, one gamete is larger in size with long slender projections and contains nearly all the mitochondria, while the other gamete is small and contains all the plastids. The two sperms of the three-celled pollen grain are usually linked by plasmodesmata, and also, one of the sperms remains joined by cytoplasmic connections to the vegetative nucleus to form a three-celled *male germ unit*.

The formation of male germ unit is of universal occurrence in all angiosperms. The male germ unit travels through the pollen tube as one unit and on reaching the ovule, the vegetative nucleus separates from the sperms, followed by separation of the two sperms.

Unusual types of pollen development

In majority of angiosperms, the pollen mother cell divides by meiotic division forming four haploid daughter nuclei, each of which then functions as the functional pollen grain. However, deviations from this normal type of development have been observed in many species. Some of them are discussed here.

1. Pollen development in Cyperaceae. In members or Cyperaceae, PMC divides meiotically to form four haploid nuclei, out of which a single functional nucleus remains in the centre and the three non-functional nuclei are pushed to one side of the cell. Functional nucleus produces the vegetative as well as the generative cell, whereas the non-functional nuclei eventually degenerate.

2. Pollen grain develops embryo sac (Ne'mec phenomenon). A single pollen grain usually contains two or three nuclei. However, in some members of Liliaceae, pollen grains with more than three nuclei have been observed. These abnormal types of male gametophytes lead to the formation of female gametophytes or embryo sac-like structures. Embryo sac-like pollen grains had been first observed in the petaloid anthers *of Hyacinthus orientalis* by Nemec.

In *Hyacinthus*, some of the microspores enlarge, escape from the spore wall and form sac-like structures. The nucleus of these sac-like structures divides by three mitotic divisions forming eight nuclei which organise themselves like an eight-nucleate embryo sac. An interesting feature of these pollen embryo sacs is that if they are placed along with normal pollen grains, or on an agar medium, the pollen tubes formed by normal pollen grains showed chemotropic response towards the pollen embryo sacs.

3. Pollen grains develop into sporophytes. Guha and Maheshwari (1966) working on *Datura innoxia* succeeded in developing sporophyte from pollen grain. It has been suggested that the pollen grains have the potential of adopting one of the three developmental procedures, e.g.

(i) They form male gametes under normal conditions.

(ii) They form female gametophytes under special conditions.

(iii) They grow into apogamous sporophytes under appropriate nutrient culture medium.

4. **Pollen grain sterility (Male sterility).** In nature, some individuals in a crop remain unable to produce fertile pollen. This condition is known as male sterility. Male sterility has been found in maize, wheat, sorghum, barley, cucumber, carrots, tomatoes and onion. Male sterility may be due to a recessive gene in an organelle other than nucleus (cytoplasmic male sterility) or due to interaction of both nuclear and cytoplasmic genes. Plant breeders have successfully used male sterility for the production of hybrids especially in sunflower,