UNIT 12 CURRENT TRENDS IN DEVELOPMENTAL STUDIES

12.1 INTRODUCTION

In flowering plants, development commences with the growth of the zygote into embryo, that is enclosed in a seed. Intensive embryological investigations during the last four decades as presented in the Units 1-6 of Block-1, have provided some insight to understand and analyse various embryological processes and compare and correlate them. However, the factors and various metabolic changes that influence the specialization of embryological structures have mostly been unresolved.

With the advent of polarizing, phase contrast, fluorescence, electron (TEM, SEM) microscopy and interference (Nomarski) contrast optics, and cyto- and histochemistry, together with the application of complementing modern cell biology techniques such as: immunocytochemistry, immunofluorescence and video-image processing it has become possible to unravel and characterise some fine structural details of the components involved in embryology. These have added several new dimensions to investigations on the structure and function of reproductive organs and tissues.

During the last decade therefore there has been a surge of information on the complex array of interrelated processes constituting reproductive biology. The implications of these investigations are likely to enhance our understanding concerning sexual reproduction in flowering plants.

Some of the major achievements that have added to our knowledge in specific areas are described in this unit to familiarise you with the current trends in this field of study.

Before you begin your study of this unit, it would be useful to brush up your memory regarding what you have studied in Units 1 to 6. Combine your prior knowledge with the information provided here to get a clearer picture of sexual reproduction in higher plants.

Objective

After studying this unit you should be able to :

- understood the finer details of the embryological process in higher plants;
- interpret the use of modern techniques in solving the intricacies of reproductive structures of angiosperms;
- explain why it is essential to adopt other relevant techniques to unravel the finer details of the cellular components;
- integrate and assess the various steps involved in reproductive biology.

12.2 POLLEN BIOLOGY

As you know from your study of Unit-1 that during microsporogenesis deposition of callose around the microspore tetrads takes place. This phenomenon is reported in several taxa. The information regarding the mode and nature of deposition of callose has been made known to us largely by the use of fluorescence microscopy. Now it has been established that callose synthesis occurs in two phases between the persistent primary wall and the cytoplasm of the sporocyte. Beginning at the pachytene stage it continues in the form of two layers till prophase I. During telophase, callose develops afresh and forms layers 3 and 4. Finally, during cytokinesis 2, more layers develop of which layer 6 causes the separation of each microspore.

In addition, it has been found that in species with simultaneous cytokinesis four or six plates form a unit while in successive cytokinesis only three plates form the unit. The plates also show positive reaction to proteins, reducing substances, and polyphosphates, revealing the complexity of composition.

Fluorescence microscopy coupled with biochemical analysis has helped resolve the differential activity of the enzyme, β-1, 3- glucanase that catalyses the dissolution of callose. The enzyme activity is relatively low during the first meiotic division but reaches its peak at the time of spore release. This sequence of events controlling the enzyme activity is important, as an early dissolution of callose may lead to sterility. Use of autoradiographic and fluorochromatic techniques demonstrated the reduced permeability of the callose wall and the resultant chemical isolation of microspores. A combination of phase-contrast and fluorescence microscopy also revealed that the callose wall during exine formation serves as a mold or template.

Factors that trigger the onset and regulation of meiosis in anther and ovule can be worked out by employing labelled reagents. Biochemical studies through isoenzyme markers have already demonstrated specific stages of meiotic process. There is an increase in the activity of acid phosphatase at zygotene, followed by subsequent decline. At pachytene, marked activation of endonucleases is recorded. Isoperoxidases serve as markers in normal pollen development in *Mercurialis annua.*

Microcinematographic techniques clearly bring out the structure of thin-walled, transparent pollen grains, contents of pollen grains including their circulation and rotation, microstructure of pollen tubes, velocity and character of protoplasmic streaming, relative movement of nucleus of vegetative and generative cell, division of generative cell and movement of the two male gametes. The information would be interesting with respect to the changes accompanying the induction of androgenesis in vitro as that would indicate involvement of different components of pollen grain.

Additional evidences suggest that considerable cytoplasmic reprogramming takes place during generative cell ontogeny. In *Gasteria*, the amyloplasts become polarised and remain entirely within the vegetative cell during the first pollen mitosis so that the generative cell inherits only the mitochondria. There may be an

other polarisation of the organelles prior to second mitosis effecting unequal distribution, for example, generative cell of *Plumbago* carries both plastids and mitochondria.

Depending upon the number of cytoplasmic organelles (mitochondria and plastids) it is possible to distinguish the two male gametes in a pollen tube. This information, and by looking at the composition of components of cytoplasm of the post-fertilization products, it is possible to predict and demonstrate the male gametes involved in syngamy and triple fusion.

The phase-specific changes in gene expression become evident as the generative cell shows period-specific changes in shape, and interactions with the vegetative cell. It is important to note that the polarization of the organelles occurs prior to first and second pollen mitosis when generative, and sperm cells are formed respectively.

It has now become possible to isolate the generative cell in living condition and study the morphological changes. The change from natural spindle shape to spherical is dependent on osmolarity of the isolation medium.

EM studies suggest that wall formation around the generative cell is not a special one but corresponds to that in other cells. The presence of callose in generative cell is transitory.

The adaptation of recent techniques like ultra-thin serial sectioning, isolation of live sperms and computer-aided 3-D reconstruction has helped unravel the finer details of the 3- celled male germ unit (MGU). The studies have produced evidence that in the majority of angiosperms the MGU is organised in the pollen tube and it travels through the pollen tube as one unit. Upon reaching the ovule, the vegetative nucleus dissociates first and the sperms thereafter.

The presence of a cytoplasmic projection of one sperm cell (*Svn*) that is superficially associated with the vegetative nucleus in the pollen tube is also evidenced from three-dimensional reconstructions based on serial thin sections. It is linked to Svn by a common cell junction. Likewise, the three-dimensional reconstruction of sperm cells of *Rhododendron laetum* and T. *macgregoriae* shows that they are paired together and both have extensions that link with the tube nucleus, forming a male germ unit. Video-image processing has revealed the presence of an axial micro-tubic cage in generative cells. The sperm cells differentiate within the pollen tube about 24 h after germination in vitro. The relevance of such a complicated male germ unit in fertilization process is presently not understood.

In sperm cells the nucleus has a densely-stained granular chromatin packed around the periphery, usually with less dense regions as nuclear vacuoles. Relatively few pores are present in the nuclear envelope as compared to the vegetative nucleus.

Of the two sperm cells, one has a long extension and is associated with the vegetative nucleus (sperm *Svn*). It carries a significantly larger number of mitochondria, than the other sperm (*Sua*). The *Sua* sperms in *Brassica* have a lower content of spherical mitochondria; infact the lowest reported for any angiosperm cell. Where both mitochondria and plastids are present in the sperm cells, *Sua* has large number of plastids. These quantitative differences once again show varying degrees of differential gene activity. The evidence for such dimorphism in sperm cells is either lacking in other cases or needs to be worked out .

It has now become possible to study pollen tubes and isolate live sperm cells in barely. The isolated sperm cells examined under Nomarski interference optics show compact cytoplasm and a conspicuous nucleus with condensed chromatin. The male gametes remain in contact with each other without showing any directional movement. The mode of relative movement of the two sperm cells to their respective destinations, the egg cell and the central cell, is still a speculation. The sperm cells change from spindle to spherical and back to spindle shape. These changes are related to cytoplasmic microtubules present around the periphery of the sperm cells. It has been suggested that changes in shape occur even during the course of pollen tube growth through the micropyle and synergid. The cytoplasm of

the sperm cells is active with small external bulges appearing and disappearing rapidly. This may indicate an interactive role related to their mobility. Thus, researches on such lines may ultimately help in our understanding towards the entire fertilisation process hitherto least understood. The cytoplasmic sheath of the sperm cell enters the egg apparatus at the time of pollen tube discharge. It is suggested that further information about the sperm cells may result if their activity is studied in the pollen tube 'sap' or embryo sac 'juice', or a combination of both.

Calcoflur is a fluorescence brightener used for detecting cellulose.

TEM studies show that the two sperm cells of *Plumbago* are connected by a calcoflur-positive cell wall transgressed by plasmodesmata. A microfibrillar ephemeral cell wall appears 15 min. after pollen germination in barely; absent in mature pollen and in pollen tubes. The sperm cells of *Brassica campestris* and *B. oleracea* are connected by interdigitating finger-like cytoplasmic evaginations. The importance of acquiring such a complex structure and arrangement needs to be worked out. Rapid-freezing and physical fixation procedures, such as, freeze-substitution will help determine the presence or absence of a periplasmic matrix component.

Recent researches in biochemistry reveal that during the development of pollen grains several haploid genome-specific genes are expressed that control pollen-specific functions, such as, pollen development, germination, sperm formation, and also stigma-style recognition. Further progress in understanding these processes can be achieved by isolating the genes that are expressed in pollen.

Recent studies on molecular genetics have thrown light on the role of microspores in controlling germination and regulating the metabolic processes. That the haploid genome is involved in transcription and translation during pollen development is evidenced from studies of several dimeric enzymes. The alcohol dehydrogenase gene (Adh 1) in maize specify a dimeric enzyme responsible for the activity of the pollen grain. Synthesis of the Adh enzyme depends solely on the genotype of the pollen nuclei and not influenced by the genotype of the diploid plant. The mRNA identified in mature pollen seem to be metabolic genes involved in tube growth. It would be challenging to determine the function of the pollen-specific genes that also control microsporogenesis.

The mature pollen grains of several species have mRNA synthesized prior to anthesis and in cell-free translation systems, code for similar polypeptides that are synthesised during germination and early tube growth. Biochemical experiments show that the mRNAs consist of three abundant classes: the first present in 26,000 (*Tradescantia paludosa*) and 32,000 (*Zea mays*) copies, the second are intermediate in number, and the third have 100 to 200 copies per pollen grain. In both the plants, the mRNAs in mature pollen grains are products of about 20,000 different genes. Based on colony hybridization, it is estimated that about 10% and 20% of the total genes expressed in maize and *Tradescantia* respectively might be specific to pollen. Zin 13 gene represents very few copies in maize genome but its specific mRNA has been demonstrated in the cytoplasm of the vegetative cell and throughout the pollen tube cytoplasm of the vegetative cell and also throughout the pollen tube after germination. Thus, it is a product of the vegetative cell nucleus. Such characterization of cell related mRNA and their product and finally their interaction with other components of the male sex structures will elucidate the nature and significance of male germ unit.

12.3 INCOMPATIBILITY

EM studies have established the concurrent development of Ü bisch bodies and sexine. The detection, identification and precise localization of exine and intine proteins became possible by employing fluorochrome-induced fluorescence and immunofluorescence. Now that so much information has become available, the two substances in both wall layers can be easily localized. The development of pollen

wall and simultaneous incorporation of proteins has also been worked out by using the same technique.

Specific regions in pollen wall have been identified which predominantly have esterases that are considered exine markers and acid phosphatases as intine markers. Hydration effect causes the release of esterases, amylases, galactosidases, glucosidases and phosphatases. These enzymes and other proteins account for pollen allergy.

It is already possible to use isoenzyme patterns of esterase and leucineaminopeptidase for the identification of maize inbred lines. The complex incompatible interactions require additional efforts to explore the possibilities of unraveling the mechanism involved. For that would help solve many unsuccessful breeding programmes. Already, enzyme and protein marking systems are being employed to find out the interrelationships between male and female reproductive structures. It has been reported that the glycoproteins related to S alleles 1,2,3,6 and 7 isolated from style extracts of *Nicotiana alata* are ribonucleases. Ribonuclease activity has been implicated in the mechanism of gametophytic self incompatibility. It has been possible to generalize that stylar fluid in hollow-styled species show esterase and phosphatase and solid-styled taxa, in addition, show peroxidases and proteases.

Some recent biochemical genetic approaches have contributed significantly towards our understanding of the phenomenon of self-incompatibility, especially in *Brassica*. The accumulated data, however, shed little light on the relationships between sporophytic and gametophytic systems. It is interesting nevertheless, that the *Brassica* and *Nicotiana* genes appear to have similar patterns of expression in cells that occupy the path of growing pollen tubes.

Plant transformation experiments are now showing promise in the analysis of different aspects of the self incompatibility response.

A relationship has been worked out between the protein kinase of maize, to the S-locus glycoproteins. The enzymes are either serine/threonin-specific or tyrosine-specific. A complimentary DNA clone from *Zea mays* encoding a putative serine/threonin- specific protein kinase structurally related to the receptor tyrosin kinases have been identified. This protein kinase is linked through a trans-membrane domain to an extracellular domain similar to that of glycoproteins encoded in the self- incompatibility locus of *Brassica* involved in the self recognition system. The identification of gene sponsored compounds involved in the incompatibility mechanism may ultimately help in resolving the intricacies of self- incompatibility.

12.4 FEMALE GAMETOPHYTE

The deposition of callose in the nucellar cells became evident with the use of modern techniques. During megasporogenesis the walls of the nucellar cells adjacent to the chalazal megaspore between the embryo sac and hypostase show callose. The amount of callose deposition increases gradually. The functional significance is not well understood. Callose deposition has also been detected in the walls of the endothelial cells. The mode of callose appearance suggests that the cells might be involved in the translocation of metabolites. A similar role can also be assigned to the nucellar cells.

Antipodals, the cells at the chalazal region of the embryo sac have been speculated to have a nutritional role. Details of structural organization that establishes this fact have been worked out through ultrastructural and histochemical methods. The antipodals have abundant mitochondria, plastids, multicisternal dictyosomes and a large number of small vesicles derived from the ER or dictyosomes. The cells are reported to have papillate wall ingrowths that appear like filiform apparatus of synergids. The wall between the antipodals and with the central cell are interspersed

with plasmodesmata. The cells are rich in oxidases, ascorbic acid, sulfhydril compounds, starch, lipid and proteins.

The presence of projections in the synergids was long established. EM studies have now revealed that the filiform apparatus of synergids is not a simple finger-like projection of the wall into the cytoplasm. Each projection has a core of tightly packed microfibrils enclosed by a non-fibrillar sheath rich in polysaccharides. Infact, the filiform apparatus resembles the spongy wall of the 'transfer cells'.

Isoenzymatic studies indicate differences in specific female tissues. However they do not point to any conclusive explanation. It is interesting that in *Lilium regale*, higher levels of glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) occur in ovary wall and inner integuments than in outer iteguments. Synthesis of insoluble reserve polysaccharide takes place in the outer integument whereas, they are hydrolysed in inner integument. The metabolites diffuse from integuments through endothelium/nucellus in embryo sac through a gradient and enzymatic action.

Recent efforts demonstrate the possibility of enzymatic isolation of embryo sacs of *Plumbago, Lilium,* and *Zea mays*. Further experimentations should be possible to assess the respective participation of different components of the embryo sac. In *Lilium longiflorum*, all developmental stages of the female gametophyte can be isolated in living conditions. Recovery of viable eggs and central cell of *P. zeylanica* will make them amenable to physiological and biochemical studies, an advantage hitherto enjoyed by male gametophytic cells.

The contraction cycle of the nucleolus of the secondary nucleus is 7 min. to several hours in *Jasione montana.* In *Galanthus nivalis*, the sperm movement in central cell of the embryo sac is about 3μm per min. Such measurements further facilitate the understanding concerning the time required for the fusion of gametes with the egg, and with the secondary nucleus. The process requires about 150 min. at 20°C in a silicon fluid medium in *G. nivalis*. The nucleoli takes about 10-15 min.

12.5 ENDOSPERM

The examination of live material of *J. montana* reveals that the division of the primary endosperm nucleus is transverse, followed by the laying down a wall. The chalazal chamber divides by a vertical wall. The elongated tubular zygote is 60-80 μm long, and the growth rate is 5-6 μm per hour. The mitotic cycle of the endosperm nuclei is short as compared to that in somatic cells. It is also possible to analyse the rhythm of development of endosperm and embryo during the initial few stages. The information would help in finding out ways to prevent embryo abortion in distant hybridization.

Enzymological studies on endosperm during development point out variable activities of different enzymes. Free-nucleate endosperm cells of *Lilium regale* show high activity of 6-PGDH and G6PDH until cellularisation process begins. The most active period of nitrogen accumulation in maize coincides with a rapid increase in glutamate synthase. Even differences between the normal and opaque-2 maize endosperm can be marked by analysing isoenzyme profiles. At day 15, the normal endosperm shows proteases I and II, whereas, opaque-2 exhibits only I at both the ages.

The localisation of three isoenzymes of G6PDH in the plastids and one in cytosol of developing castor endosperm reflects that young castor endosperm is the seat of both, glycolytic and pentose phosphate pathways. The embryo-endosperm relationship and the role of endosperm during seed development is an important aspect needing further attention.

12.6 EMBRYO

The suspensor cells of young embryos of *Tropaeolum majus* show that the activity of acid phosphatase increases from micropylar to chalazal pole. The concentration of 6-PGDH and G6PDH is high in very young embryos of *Lilium regale* By globular stage the activity decreases considerably. Implicit in these results is the fact that different regions of embryo would provide some explanation of different embryogenic processes. Also, a marker system can be established for identifying differentiating areas in the developing embryos. What is needed is the uniformity of the enzyme system being explored in different climatic conditions which may affect metabolic processes thus influencing the sexual cycle. This may lead to detection of variations in developmental sequences or abnormalities so often reported in the literature.

12.7 SUSPENSOR

The structure and function of suspensor have not been given adequate attention During the last decade, however, investigations have produced noteworthy results. These include ultrastructural, isoenzymatic, physiological, and in vitro experiments. A great deal of variation occurs in suspensor structure, probably modified to support the developing embryo. During 1950's and 1960's embryologists believed that it the suspensor is merely a morphological organ that pushes the embryo deeper into the more friendly environs of endosperm. This view is gaining reconsideration. The suspensor plays a more dynamic role than was hitherto assigned. The special kind of plastids present in legumes such as *Pisum* and *Phaseolus* and in *Ipomoea* and *Tropaeolum* show remarkable ultrastructural changes around late heart-shaped stage of embryo. The significance of these unusual plastids needs to be determined.

Another feature of interest is the presence of wall embayments lined by plasma membrane in the suspensor cells supposed to be involved in short distance translocation of metabolites similar to transfer cells. Some experiments demonstrate the significance of presence of suspensor for proper development of the embryo. During early stages, removal of suspensor reduces embryo development but, at later stages, it has no effect. However, it is possible to replace, at least partially, the effect of suspensor loss by providing gibberellins in the culture medium. The finding is further substantiated by quantitative analysis of GA present in the suspensor and embryo proper cells. This GA has been identified as gibberellin A1.

The relative concentrations of auxin in embryo proper and suspensor of *Tropaeolum majus* have been studied through single ion detection. The suspensor proper yields significantly higher concentrations of auxin. Likewise, in *Phaseolus*, the suspensor of heart-shaped embryo shows more cytokinin. However, at mid-cotyledonary stage, suspensor contains low cytokinin and the embryo seems to become autonomous for cytokinin.

These findings clearly indicate that suspensor is a reservoir of growth hormones meant for supporting embryo development. Whether these hormones are synthesized de novo in the suspensor cell or it acts merely as a conduit remains to be answered.

12.8 THE FUTURE

Fertilization in flowering plants is essential for sustaining life on earth. Production of most crops depends on the effectivity of the fertilisation process. The emergence

of new cultivars and plant improvement until recently depended exclusively on normal fertilisation. Therefore, the active involvement of a number of research techniques bears testimony to the fact that reproductive biology is emerging as one of the most exciting areas of plant biology.

During the past decade major emphasis has been to understand the male gametophyte and the mechanism of incompatibility as these are crucial for the success of breeding programmes. The last five years have witnessed a surge of information on identification and control of haploid genome regulated activities during initial pollen germination and fertilisation. Characterization, and structure of sperm cells and structure and composition of pollen tubes are also attracting considerable attention. Immunofluorescence techniques confirmed the presence of proteins in the pollen walls which enhanced our understanding of the acceptance and rejection (incompatibility) response. Immunological principles would help explain the nature of the product of the sporophyte cells involved in gametophytic and sporophytic incompatibility.

Isolation of entire embryo sacs and its individual cells opens up new possibilities for the study of fertilisation process and embryo-endosperm relationship. Some ingenious experimental studies with suspensor demonstrated its significance during early stages in providing growth support to the developing embryo. Labelling studies may finally reveal the site of biosynthesis of nutrients and growth hormones and the creditable role of the suspensor.

With the aid of computerised imaging, the structure of sperm cells would become more clear, as yet incompletely understood.

The immensely growing body of knowledge of various embryological structures mainly from biophysical and biochemical investigations have provided added impetus for probing these structures in living state. Identification and localization of various cellular constituents therefore acquire additional significance. A creditable method capable of analyzing biochemical events in single cells or small aggregates will provide ample details and thus answer to a myriad of questions.

In embryological studies, now the principal criteria should be an estimation of the rate of success of any individual plant in parenting viable embryos. Towards this goal, together with several new techniques, embryologists can contribute extensively in making quantitative estimates of integral components of sexual process. This can also be used for a functional analysis of reproductive behaviour in any flowering plant, including tests of the capacity of the male or female organs involved in reproduction process.

It is thus surmised that rapid progress in techniques and consequently in approach has yielded valuable information in the area of angiosperm embryology. Further efforts will benefit our knowledge and enhance our understanding of various aspects of plant embryology hitherto unexplained.

12.9 SUMMARY

The study of this unit has acquainted you with the recent findings in reproductive biology, particularly in the fields of pollen biology; incompatibility reactions during sexual reproduction; several functional aspects of female gametophyte, endosperm, embryo and suspensor. The deployment of various modern tools and techniques have answered several questions that have been arising in the minds of biologists for a long time. The coming time holds great promises and it is going to be very exciting one; as clearer picture of plant development is likely to emerge. his has enormous application in the production of novel plants and in the improvement of existing plants.

12.10 TERMINAL QUESTIONS

1. Fill in the blank spaces with appropriate word (s).
   1. The ..... wall surrounding the microspore serves as a mold or template during the formation of exine.
   2. In the pollen tube, one of the sperm cells showing a large cytoplasmic projection is associated with the. . . . . .
   3. The change in shape of the sperm cells from spindle to spherical and back to spindle shape is controlled by the ..... present along the periphery of the sperm cells.
   4. For studying the pollen wall, ... are mostly used as markers for exine and ..... as markers for intine.
   5. The filiform apparatus of the synergids is similar to the spongy wall of the ..... cells.
   6. For the proper development of embryo, the presence of ..... is necessary.
2. State which of the following statements are not true?
   (i) The synthesis of callose around the microspore tetrads occurs continuously.
   (ii) The microcinematographic technique enables us to study the following: the structure of thin walled pollen grains; microstructure of pollen tubes; velocity and character of plasma streaming, division of generative cells.
   (iii) The two male gemetes in a pollen tube differ from each other with respect to some of their cytoplasmic organelles.
   (iv) Several haploid genome-specific genes are expressed during the development of pollen grains, that control functions such as pollen development, germination, sperm formation etc.
   (v) By the application of modern techniques it has been established that the antipodals prevent the downward growth of the embryo sac.
   (vi) The filiform apparatus present in the synergids is composed of tightly packed microfibrils that are enclosed by a polysaccharidic sheath.
   (vii) Like the male gametophyte, viable embryo sac and its components can be isolated.
   (viii) The suspenser is a reservoir of growth hormones, and it supports the proper development of embryo.
3. During which time of microspore development, is the activity of enzyme β-1, 3-glucanase at its peak?
4. What would be the consequences, if the enzyme β-1,3-glucanase shows its maximum activity, say during meiosis-1?
5. What is the fate of 3-celled MGU (male germ unit): (i)while in the pollen tube, and (ii) on reaching the ovule?
6. Give two features, on the basis of which we can differentiate between the two sperm cells (*Svn* and *Sua*)?
7. What components of pollen wall cause allergenic reactions in humans?
8. Which characteristics of antipodal cells indicate that they perform a nutritive role?
9. Comment on the following statement: 'the mitotic cycle of the endosperm nuclei is short as compared to the somatic cell'.

10. What facts have been brought to light by the modern investigations on the functional role of suspensor?

11. Combining your prior knowledge on reproductive biology of angiosperms from your study of Block-1, and what you have learnt in this unit, prepare on update write up on the following aspects : (i) microspore development; (ii) : incompatibility; female gametophyte; the roles of endosperm and suspensor.

12. Prepare a list of various techniques mentioned in this unit?

12.11 ANSWERS

Terminal Questions

1. 1. callose
   2. vegetative nucleus
   3. microtubules
   4. esterases, acid phosphatases
   5. transfer
   6. suspensor
2. (i)
   (v)
3. At the time of spore release.
4. This may lead to sterility.
5. (i) It remains as one unit, as they are connected through their extensions.
   (ii) The vegetative nucleus dissociates and the sperms separate later on.
6. The *Svn* sperm cell has long extension and is associated with vegetative nucleus, and contains a large number of mitochondria than the other sperm.
7. Esterares, amylases, galactosidases, glucosidases, phosphatases and some other proteins.
8. Hint: they have abundant mitochondria, plastids, multicisternal dictyosomes, small vesicles derived from ER or the dictyosomes, wall ingrowhts like synergids, walls between the antipodals and synergids have plasmodesmata, are rich in oxidases, ascorbic acid, sulphydril compounds, starch, proteins and lipids.
9. Hint: The endosperm has to develop faster to provide nourishment to the developing embryo.
10. Hint:The old view has gained firm footing that the suspensor pushes embryo deep into the nutritive medium - the endosperm. The presence of growth hormones like cell auxins and cytokinins; and unusual plastids, presence of wall embayments lined by plasma membrane suggest their role in short distance translocation of metabolites like the transfer cells.
11. You may refer to the related portions in Units 1-6 and the present unit.
12. Some of the techniques mentioned in this unit are: Fluorescence microscopy, Biochemical analyses, Microcinematography, Ultra-thin section, EM studies, Video image proceeding, Nomarski interference optics, Freeze substitution, Immunofluorescence, and Histochemical techniques.