

EXERCISE 23 ANGIOSPERM EMBRYOGENY: DOUBLE FERTILIZATION AND EMBRYOGENESIS

Pollen tube growth. Growing pollen tubes is simple if you are using bicellular pollen. Physiologically, it is possible to grow many pollen grains on a fairly simple Brewbaker-Kwack medium (0.01% H_3BO_3 , 0.03% $Ca(NO_3)_2 \cdot 4H_2O$, 0.02% $MgSO_4 \cdot 7H_2O$ and 0.01% KNO_3), with from 0% to 20% sucrose added. Obtain a slide and place a small drop of pollen growth solution on it. Then sprinkle some fresh pollen on the drop. Wait for a minute or two for the pollen to settle into the solution and hydrate. Then, VERY CAREFULLY add a cover slip. (Hydrated pollen grains break easily!) Now, to prevent the slide from drying, either put it in a humid chamber or seal the edges of the cover slip by applying liquid Vaseline to the outside edges of the cover slip. (A warm paper clip is an ideal applicator, if used carefully.) Pollen tubes should form within 10-30 minutes. These will be visible using a compound microscope or high magnification with a dissecting microscope. For showing rapid growth, pollen of *Tradescantia* and *Impatiens* is excellent. To show the movement of the generative cell down the pollen tube, *Amaryllis* is ideal. As these elongate, can you see the vegetative nucleus? the generative cell? a linkage between these structures? When are the sperm cells formed?

Male germ unit. The male germ cells (either the sperm or generative cells, depending on the pollen grain and stage of development) are typically associated with the vegetative nucleus prior to germination and during pollen tube growth. This association has been termed the *male germ unit*. Examples are in the lab. Although the exact form of the MGU varies from one species to the next, this association may be important in delivering the two gametes into the embryo sac at the same time, preventing *heterofertilization* (a condition in which sperm cells from different pollen tubes fertilize the egg and central cell.) The two sperm cells are linked by retaining a common cell wall, as a result of generative cell cytokinesis and both are contained within an internal vegetative cell plasma membrane. A second physical association occurs between at least one of the sperm cells and the vegetative nucleus. This forms a more or less intricate arrangement of complementary surfaces that is maintained up to the time of fertilization. The grasses are exceptionally interesting in this regard, because their association is severed early in development, but re-established just prior to fertilization. This argues strongly for the importance of the MGU. This association appears to occur in the vast majority of angiosperms.

Fertilization. The function of the mature embryo sac is a complex process that can be divided into four stages:

1. **Progamic phase** - A preparative stage in which the male and female gametophytes are slowly modified prior to arrival of the pollen tube. This includes, for example, the degeneration of the receptive synergid
2. **Gamete discharge** - Arrival and discharge of the pollen tube, constituting sperm deposition
3. **Gametic fusion** - fusion of plasma membranes of the gametes, transmitting the nuclei of the male gametes into the egg cell and central cell
4. **Nuclear fusion** - fusion of the nuclear envelopes of the male and female gametic nuclei

Demonstrations are available on each facet of the process. The exhibit centers on results using *Plumbago zeylanica* and *Populus deltoides*, two plants in which these processes have been clearly elucidated. Several alternative models are also shown in another figure on display.

Embryogenesis. After the formation of the zygote and primary endosperm nucleus, considerable development is usually required to organize the mature seed. Endosperm development, which creates the nutritive material for the embryo, usually precedes the division of the zygote and further embryogenesis. The processes of embryogenesis and endosperm development are illustrated in *Capsella bursa-pastoris*, shepard's purse, which is the classical plant for this research (and is similar to *Arabidopsis* in its pattern of development). Slides are available illustrating the organization of the young embryo, including:

1. globular embryo stage - the first stage in the formation of a three-dimensional cell division system producing tissue thickness in the embryo. Note the long suspensor and unusual basal cell, which is found in many members of the Brassicaceae. What is the function of the suspensor?
2. heart-shaped stage - differentiation of the early cotyledons. This stage also shows the first conspicuous evidence of a protoderm. What is the developmental potential of that layer?
3. torpedo stage - elongation of the embryo. The embryo becomes more complicated at this stage and the apices are becoming organized.
4. maturational stage - growth of the embryo is completed within the sclerified ovular integuments, forming the seed.

One caveat--Capsella has a curved megagametophyte (ana-amphitropous), which is a highly restricted characteristic in angiosperms. The events are given in detail for *Capsella* in your text.

A remarkable reduction of the embryo is shown in the embryo of *Monotropa uniflora*, the parasitic Indian pipe. Embryos of this plant are only two cells at maturity. In angiosperms, there is wide variation in the organization of the embryo in different groups.

As a last exercise on the organization of embryos, examine the mature embryo of *Zea mays*, as a representative of the monocots. The embryo of corn is particularly precocious compared to most flowering plants. Observe a prepared slide and identify the scutella, shoot apex, root apex, epiblast, coleoptile, coleorhiza and young foliage leaves. What constitutes the cotyledon? Is there any evidence that a second cotyledon was ever present in monocots?