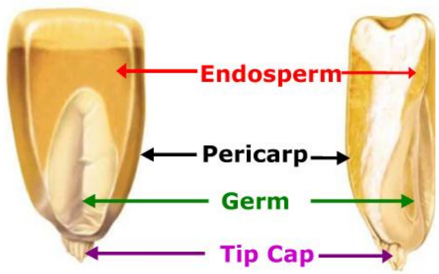
Endosperm culture

In angiosperms the endosperm is the main nutritive tissue for the embryo. The endosperm is the product of **double fertilization** during which out of the two male gametes, one fertilizes the egg to form zygote and other fuses with secondary nuclei to form triploid endosperm. Hence, triploid nature of endosperm is the characteristic feature of angiosperms. Both mature and immature endosperm can be used for culture initiation. A key factor for the induction of cell divisions in mature endosperm cultures is the initial association of embryo but immature endosperms proliferate independent of embryo. The endosperm tissue often shows a high degree of chromosomal variations and polyploidy. Mitotic irregularities, chromosome bridges and laggards are the other important characteristics of endosperm tissues. Triploids are usually seed sterile and is undesirable for plants where seeds are commercially useful. However, in cases where seedlessness is employed to improve the quality of fruits as in banana, apple, citrus, grapes, papaya etc. the induction of triploid plants would be of immense use. Triploid plants have more vigorous vegetative growth than their diploid counterparts. Hence, in plants where the vegetative parts are economically useful, triploids are of good use.

The endosperm is a homogenous mass of parenchymatous tissue lacking in vascular elements. Since the endosperm tissue lacks in differentiation into specialised tissue and vascular element their utility in the study of experimental morphogenesis is well appreciated. Attempts to grow endosperm under *in vitro* condition dates back to 1930 by the scientist Lampe and Mills. The young corn endosperm was cultured on the extract of potato or young corn by the above scientist and slight proliferation of tissue was noticed from the tissues surrounding the embryo. In 1947, LaRue, successfully cultured the corn endosperm and obtained plantlets with root-shoot axis and miniature leaves. Several investigators, since then, have cultured the endosperm tissue but invariably have failed to induce organogenesis. However, successfully organogenesis was achieved from endosperm callus tissue of *Ricinus communis, Oryza sativa* and *Pyrus malus*.

Corn endosperm



Callus induction from endosperm

During the early period of endosperm culture, there was difficulty in establishing callus induction from mature endosperm. Of late successfully regeneration of triploid plantlets are being achieved. The process of regeneration may be direct from the endosperm or *via* callus stage.

Callus tissue is induced from the endosperm explant in usual manner as with other explant. The endosperm tissue is homogenous in nature surrounded by a single peripheral layer of meristematic cells. These meristematic cells undergo repeated periclinal and anticlinal divisions resulting in increased girth of endosperm tissues and in turn producing callus with nodular structures on the surfaces or just below the outer most layer. The plants, which have so far responded favourably, belong to the families of *Euphorbiaceae, Loranthatceae and Santalaceae*. With respect to members of first two families the embryo has to be maintained intact along with endosperm in culture, to induce the callus from the endosperm. In these cases, immediately after the callus induction from the endosperm the embryo should be removed under aseptic conditions to avoid the formation of embryo-endosperms callus mixtures. The essentially of the initial association of the embryo endosperm for inducing proliferation is that during germination of the embryo, it releases gibberellin - like substances which turn in help in *de novo* synthesis of other enzymes responsible for the endosperm proliferation. These substances are otherwise called `embryo factors'.

The age of endosperm is critical factor influencing proliferation of endosperm tissue. Endosperm cultured immediately after pollination normally do not proliferate. In crops like rice, maize, endosperm proliferates 7 to 8 days respectively after pollination. In many cases, endosperm of cellular nature proliferates more easily than nuclear endosperm or coenocytic endosperm. In some species the culture of endosperm with their embryo produced favourable condition for the proliferation of endosperm whereas in species like *Taxillus verstitus*, culture of endosperm after cutting it into two species produced better results. In endosperm culture, the proliferation thus induced occurs at different duration and it is a genotype dependent phenomenon. For example, the endosperm of *Ricinus communis* proliferated 10 days after culture whereas the endosperm of *Pyrus malus* and *Santalum* took 15 to 21 days respectively for proliferation.

Morphogenesis of endosperm callus

Straus (1954) stated that the endosperm tissue has passed through approximately 95 transfers and has produced an estimated 15 kilograms of tissue. Not a single example of complex differentiation was observed during the period. However, both organogenesis and somatic embryogenesis were observed, in other cases. The first convincing evidence of organ formation was from *Exocarpus cupriformis*. The incidence of organogenesis from the above species was noticed in the form of shoot buds all over the surface of the endosperm. Apart from the direct organogenesis in the form of buds, the organogenesis may also follow the pathway *via* the callus stage. The formation of shoot buds increased with increase in cytokinin concentration and decrease in auxin concentration. In general, endosperms of all the plant species showed increased bud formation with response to higher concentration of cytokinin. The studies on the role of auxin (IAA) and cytokinin (kinetin) revealed that cytokinin alone was more effective in combination with auxin: there was no differentiation in the absence of cytokinin, but cytokinin is not always necessary to induce bud formation from normal tissue. But for endosperm tissues to produce buds cytokinin is required. Presence of organic additives like tomato juice, coconut milk, casein hydrolysate, yeast extract in the culture medium enhanced endosperm proliferation and regeneration.

Application of endosperm culture

The cultured endosperm forms an excellent experimental system for physiological and morphogenetic studies. This system shows great promise in the study of metabolism and differentiation. The triploidy can be exploited in the crops *viz*. apple, banana, mulberry, sugarbeet, tea and watermelon where seeds are not of commercial importance. In some plants especially in clonally propagated ones triploids are superior to the diploids giving better pulp woods. Since these plants can be propagated vegetatively seed sterility is not a severe setback. In the case of conventional method of triploid production, crosses are made between auto tetraploids and diploids. Sometimes, these crosses may not be successful causing difficulty in triploid production.

Nature of impairments in embryo development and causes

Causes	Source	Example
Normal initial rate of growth followed by retardation in later stage	Embryo	*Oenothera biennis* x *O. muricta* *O. biennis* x *O.lamarkiana*
Paucity of cell organelles due to lower synthetic capacity of hybrid genome, necrosis and dumping	Embryo	*Hibiscus costatus* x *H. aculeatus* *H. costatus* x *H. furcellatus*
Hybrid failure due to pollen incompatibility	Pollen incompatibility	*Pinus pence* x *P. cembra* *P. strobus* x *P. flexilis*
Disintegration of endosperm soon after fertilization	Endosperm	*Oenothera* and *Gossypium*
Vacuolation in endosperm cells at chalazal and not followed by further cell division	Endosperm	*Lycopersicon pimpinellifolium x L. peruvianum*
Abnormal behaviour of antipodals and preventing nutrient supply to embryos	Endosperm	*Citrus (2x) / Citrus (4x)* *Gossypium hirsutum x G. arboreum*
Intrusive growth of somatic tissue causing somatoplastic sterility	Nucellus	*Nicotiana rustica* x *N. tabacum* *N. rustica* x *N.glutinosa*
Non-differentiation of integumentary cells into connective tissues to connect main vascular bundle with chalazal tissue	Integuments	*Nicotiana* hybrids

Examples of wide crosses

Wide crosses	Purpose
Corchorus capsularis x *C. olitorius*	Hybrids had fibres with quality of *C. capsularis* mand strength of *C. olitorius*
Hordeum vulgare x *H. bulbosum*	The hybrids possessed winter hardines and mildew resistance like *H. bulbosum*
Lycopersicon esculentum x *L. peruvianum*	The hybrids possessed resistance to viruses,molds and nematodes along with good fruit set like *L. peruvianum*
Melilotus officianalis x *M. alba*	Hybrids resembling *M. officinalis* in agronomic characters and low coumarin content like *M. alba*
Nicotiana tabacum x *N. resophilia*	To get plants with resistance to black shank
Oryza sativa x *O. officinalis*	To transfer pest resistance
Trifolium pratense x *T. sarosiense*	To impart perennial plant habit to red clover

Questions

1. Endosperm is …………… in nature

a). Triploid b). Tetraploid c). Monoploid d). Diploid

2. Endosperm is product of …………… fertilization

a). Double b). Single c). Triple d). None of the above

3. Triploid nature of endosperm is the characteristic feature of

a). Angiosperms b). Gymnosperm c). Pine d). None of the above

4. The important characteristics of endosperm tissues are ………..

a). Chromosomal variations b). Polyploidy

c). Chromosome bridges and laggards **d). All the above**

5. Successfull organogenesis was achieved from endosperm callus tissue of ………..

a). *Ricinus communis* b). *Oryza sativa*

c). *Pyrus malus* **d). All the above**

6. Endosperm culture is successful in ………..

a). *Euphorbiaceae* b). *Santalaceae*

c). *Loranthatceae* **d). All the above**

7. Triploidy is exploited in ………….

a). Apple b). Banana

c). Watermelon **d). All the above**

Additional sources

http://www.youtube.com/watch?v=Gq8NWh98wQs&feature=related - video

http://www.tutorvista.com/content/biology/biology-iii/angiosperm-morphology/fertilization.php