Course Name: Basics of Crop Breeding and Plant Biotechnology

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Lecture-44: Haploids and Artificial Seeds

Welcome back. So, we will continue again. Then in-vitro haploid production we can do by anther culture also, we can do by ovule culture also. The anther culture it was reported in *Nicotiana tabacum*, in *Hyoscymamus niger*, while the ovule culture it was reported in Liliaceae family, Compositae family in those crops. So, these are the earlier reports where mature pollen grains were used to produce haploids. Now, these are the things which are available in a complete flower that is known to us, in a complete flower the male reproductive part is available it will be having anther, filament. Ok! In anther, the pollen grains are produced those are the male gamete. Ok!

While over here, in the female reproductive tissue it contains the long style, the feathery stigma in most of the cases ok, and this is the ovary part. So, within this ovary, the cell division is taken place and eventually the egg cell is formed over there. So, once this pollen and egg cell fused, then we can see the seed formation in nature, but this pollen cells and this egg cells, both are haploid ok, because both are generated through meiosis. So, if you see the different parts of the ovary over there, if this is the egg cell, these two are known as synergids, while at another pole you can see three cells, those are the antipodals and in between the polar, nucleus are available.

Now, if we see about the pollen grain formation in anther, these are the different stages within the microsporocyte; first meiosis I is taken place, then meiosis II is taken place and thereafter, in this way tetrad is formed. Ok! Eventually, in this tetrad, all of them will

be converted into pollen grains. So, these are the process of microsporogenesis. So, one pollen grains are formed, that pollen will move over here. So, this is the process of pollination and thereafter how this pollen grain eventually will develop ok, we will see little bit.

So, these are different stages. So, first of all, the released, this is the structure of released microspore, thereafter, the polarized microspore is formed in this particular pollen grain then pollen mitosis I occurs, then eventually pollen mitosis II occurs and two sperm cells are produced. So, these are the different process of microsporogenesis. Now, we will discuss the in-vitro haploid plant production. Ok! We can start the process from different parts the unopened flower bud could be taken, therefrom, the immature anther could be taken and we can make callus from there.

Similarly, from a mature anther, means the open flower, we can take the uninucleate pollen and eventually we can develop callus. Then, from the microspore, we can do microspore culture, it can convert into callus and from microspore, directly we can get the embryonic formation and from those embryonic, directly we can get the haploid plants. In rest of the cases, from this callus eventually we can develop the haploid plant. So, this is the process in a nutshell, from this different tissues or different cells eventually we can develop haploid plants. So, now coming to the factors influencing anther culture.

So, genotype of donor plants, then anther wall composition, the culture medium, the stage of the anther, the effect of temperature and physiological status of the donor plant. So, these are the things which basically influence the anther culture process. And now, we will be discussing the importance of pollen and anther culture. Some of the things we have discussed, but let us see once again, first of all, cytogenetic studies aids in observing chromosome behaviour because in anther culture, single set of chromosomes are there right in we are going to develop haploid. So, single set of chromosomes are there within the microspore.

So, we can study their behaviour, the ploidy level and abnormalities for advancing

cytogenetic research. So, if we do anther culture in polyploid species ok, therefrom we can determine the ploidy level also. Ok! Because within a cell, if too many chromosomes are there, determination of the ploidy level might be difficult. In anther culture, the chromosome number will be half and relatively, in a relatively easier way we can determine the ploidy level. If any abnormalities are there, if any chromosome is not coming in the gametes, that things, we can detect over there through cytogenetic studies. Then genetic recombination, it induces controlled genetic recombination enabling the study of new genetic combinations. Ok!

Means from here, we can identify a couple of specific gametes which could be eventually converted into double haploid and they could be crossed to get a superior combination. Ok! New genetic combination could be generated from here. Next, differentiation and organogenesis, anther or pollen cultures allow insight into cellular differentiation and organ formation process. Means, eventually, if successfully we can do the culture we can develop the plantlets, we can develop the haploid plants. Then pollen embryogenesis, these cultures help to understand factors influencing pollen embryogenesis mechanisms. Ok!

How a pollen could be converted into embryo ok, those process could be optimized. So that, if we identified pollen, then easily we can form the full-grown plants without formation of callus and those things. So, then double haploid production, we have discussed it again and again to produce fertile, genetically uniform, double haploid plants for genetic research through pollen culture or anther culture. Next one is mutation study. Ok! So, it can reveal mutations, in normal condition we may see the heterozygote condition capital A and small a, but somehow, if we get the pollen grains having small a allele by chromosome doubling we can make small a small a.

So, we can know the function of the recessive homozygous ok, and we can identify what type of mutation it is, what is its function. Then, in plant breeding it facilitates accelerated plant breeding and genetic advancement through controlled in-vitro growth of plant reproductive tissues. Then, alkaloid production, anther culture obtains alkaloid,

high alkaloid content in homozygous *Hyoscyamus niger*. Ok! So, in *Hyoscyamus niger*, high level of alkaloid production was reported in from the anther culture. Ok! So, in genetic research, the haploids from *Arabidopsis*, *Lycopersicon* that is tomato, used to transfer and express genes from *E.*

coli, means from *E. coli*, some genes have been cloned, and it was expressed within the haploids developed from *Arabidopsis* and tomato for characterization of those *E. coli* genes. Well, now we will start artificial seeds. First of all, plant embryos, shoot buds or cluster of cells from various plant tissues are carefully enclosed within a protective coating. Ok!

So, it could be plant embryo, it could be shoot bud, or a cluster of cell which is enclosed within a protective coating. These encapsulated plant structures possess the potential to develop into fully grown plants when provided with the appropriate conditions, means it is a encapsulated seed like structure. Ok! Over there the shoot bud or embryo is available and it has the ability to develop into a fully grown plant. So, either within a controlled laboratory condition or outside within the suitable environmental condition. So, the aim of artificial seeds is to facilitate the efficient propagation of plants, while safeguarding their delicate developmental stages. Ok!

So, the efficient propagation could be done suppose, some plantlets maybe in case of tobacco, some small plantlets have been formed and those things are available somewhere in Kerala and we need to transfer it into Assam. So, it will take a long time to transport it from Kerala to Assam. So, if we make such type of artificial seeds so it could be transported easily and the developmental stages will not be hampered and the successful plant could be grown. So, in this way, within the artificial seeds different things are available, we have to provide some growth regulator, we have to provide some gelling agents, then additional materials such as antibiotic or antifungal materials are given. So that, during the transport process, no contamination is taken place, then the nutrient elements should be there, because, whatever the embryo or shoot bud is available inside, it should get enough nutrients to sustain. Ok! Explained mostly, the somatic

embryo is used over there. So, this is the concept of artificial seed. So, why there is a need of artificial seeds? First of all, seed preservation and propagation. We can preserve the seeds, the orchid seeds or orchid propagules could be preserved easily and it could be transported across the country also. Ok! Then conservation of endangered species through tissue culture, we can conserve it by making artificial seeds we can conserve it and we can again grow it in the new habitat. Ok!

In the new place where it is supposed to grow, where adequate temperature or climate is available, adequate soil condition is there. Ok! We can introduce new species also. Then efficient clonal propagation means that is true for most of the tissue cultural things. So, the clonal propagation could be done or maintained through artificial seeds. Then hybrid propagation, then disease elimination if we make the disease-free plantlets by making artificial seeds, we can remove the disease, we can eliminate the disease. Then uniformity in growth, that is an important point. Generally, if we see the plantlets are growing from callus, then from different part of the callus, we can see differential growth, from some part maybe a bigger plant can grow, from some part maybe only the root tissue is start to initiate, start to grow, ok, means lack of uniformity is observed. But in artificial seeds, we can maintain such type of uniformity because, once we will send it for subsequent growth, either in the field or in other laboratory, once it will be in uniform stage, its maintenance will be easier also. Then reduce space requirement useful in research and biotechnology. So, now we will discuss the basic requirement for the production of artificial seeds.

First of all, we need vigorous somatic embryos, means large number of somatic embryos are needed and vigorous growth should be there, means if growth is very slow then we cannot produce adequate amounts of artificial seeds at a time, because we have to make large number of seeds at a time, later on I will come. Then, inexpensive production of large number of high-quality somatic embryos with synchronous maturation, few terms are there, first of all, the production should be inexpensive means not too specific hormonal conditions or too longer period of time, if needed, then we should avoid it. In those crops where inexpensive methods are there so that we can get to

develop somatic embryos which will mature synchronously within shorter period of time. So, those things will be preferred. Then, we need to optimise the encapsulation and coating system that is very important part in artificial seeds.

Then, what are the difference between artificial seeds and natural seeds? In artificial seeds, the production from asexual reproduction, right, means somatic embryo or callus those things, mass of cells, it could be converted into artificial seeds, but in natural seed, it is produced from sexual reproduction. Then artificial seed, it does not involve fusion of gametes, while, it involves male and female gamete fusion. Then, it is produced from vegetative cells, it is produced from zygote by fusion of germ cells. Then, artificial seeds contain genetic constituent of single parent, from a single parent it is being developed, here, genetic constituents are coming from both the parent male and female parent. Then, genetic recombination does not take place, as from the somatic cell it is being generated, so, we will get a clone, all genetic constitution will be same while, over there, the genetic recombination take place.

It contains only embryo and endosperm, seed coat is absent in most of the cases while, it contains all embryo, endosperm and seed coat in most of the natural seeds. Now, we are coming into the procedure of artificial seed production. So, first of all, we have to establish the proper somatic embryogenesis process, means, somatic embryo formation from callus we should optimize first. Then, we need to mature the somatic embryos thereafter, synchronization and singulation of somatic embryos is needed, means, all the somatic embryos within a plate will be synchronized and we should isolate it separately, means, if it is clubbed together, then it could not be useful for the artificial seed production, we need to singulate, we need to separate different somatic embryos. Next one, the mass production of somatic embryos, means, once this process is optimized, then we have to produce the somatic embryo in large number, then standardization of encapsulation what gel media or agarose will be used, we need to optimize it and then the standardization of artificial endosperm, means here, the embryo has been formed.

In artificial endosperm, we may put different nutrients, different growth hormones,

some antibiotics or antifungal agents. Ok! So that, the seed will be preserved for longer period of time. Next one, we have to produce the artificial seed in mass amount and finally, it will be transferred into greenhouse and field for planting. So, it is in a nutshell, the procedure of artificial seed production. So basically, two types of artificial seeds are produced; mainly one is the desiccated synthetic seed, another one is hydrated synthetic seeds. Ok!

In desiccated one, the desiccated artificial seeds can be either a naked or encapsulated in PEG i.e., polyethylene glycol before undergoing desiccation. So, either the seeds will be in naked condition or it will be encapsulated in PEG, thereafter, we have to do desiccation, means, we have to remove the excess water what is available over there. So that, it could be preserved or it could be stored for longer period of time. This process involves carefully reducing the moisture content to enable storage without active metabolism. So here, we need to minimize the active metabolism process.

Then, desiccation can occur rapidly by leaving seeds in open petri-dish overnight or more, controlled by gradually lowering humidity. Within a particular wood, we can put those artificial seeds in open petri-dish and after overnight, in overnight most of the moisture will be removed and thereafter gradually, we can reduce the humidity. So, the moisture level could be minimized further. Then, desiccation tolerance can be induced through technique like high osmotic potential in maturation medium or sub-lethal stresses and the addition of permitting osmotic agents such as mannitol, sucrose. Mannitol is mostly used for such type of work because you know, if the dehydration stress should be given in a plant under laboratory condition, we can treat it with mannitol.

Because, mannitol is one of the important chemicals which is used to provide the drought stress or it will remove the water content. The desiccation could be easily done applying mannitol or sucrose. Then coming to the hydrated synthetic seeds, the hydrated artificial seeds are created by enclosing somatic embryos with hydrogel capsules providing both protection and a transition to artificial seeds. Here, basically, hydrogel capsules are used. So, some sort of moisture should be retained over there.

This method is particularly employed for plant species that are recalcitrant and susceptible to desiccation. So, recalcitrant crops species or plant species, they are susceptible to desiccation. So, for those, mostly we need to follow the hydrated synthetic seed production. These synthetic seeds facilitate the preservation and propagation of sensitive micro-propagules in-vitro, example alfalfa, barley, sandalwood. Ok! So, in those crops, in sandalwood, it was found to be very famous, the hydrated synthetic seeds could be produced and it could be transferred from one place to another place.

Now, we will be discussing two different artificial seed encapsulation methods. First one is dropping method, next one is molding method. In dropping method, first, the preparation of encapsulation material is done. We need to make the encapsulation material mostly the calcium alginate or those things are used over there, sorry, the sodium alginate is mostly used over there. Then, we need to prepare the plant tissue sample.

Usually the somatic embryos are used over here. Then, gradually we have to use this particular encapsulation material and one by one somatic embryo will be placed over here. And once the somatic embryo will be placed, it will be encapsulated, it will be encapsulated by the encapsulating material. Thereafter, through dropping method, the droplets will be formed and it will fall down in a beaker or somewhere, where mostly the calcium chloride escape. So then, the calcium alginate formation is taken place, means gelation process is taken place and in the surface of those somatic embryos, the final gel casting is taken place.
Once that thing is formed, we need to rinse it and collect it and thereafter, curing is done. So, in this way, through dropping method, we can develop the artificial seeds one by one. While in case of molding method, here selection of encapsulation material is also done over here. Over here, in this way, a block like structure is initially prepared. Then we have to prepare the plant tissue and mold preparation is done.

Once the mold preparation is done, then gradually different plant tissues are inserted. The plant tissues are inserted over there. And then the gelation process is taken place

then we need to demold it and collect separate artificial seeds and then finally, we have to do rinsing and curing process. So, these are the two different methods of artificial seed encapsulation.

Now, let us come to the artificial endosperm. Till now, we have discussed about the artificial seed, the somatic embryo mostly used over there. In artificial endosperm, the somatic embryos basically, it lacks the natural protective seed-coat. In nature, we can see the seed coat or tester formation, but in somatic embryo, the natural protective seed coat is not there and nutritive endosperm is also not available over there. So, to address these deficiencies, the encapsulation matrix can be enhanced with nutrients and growth regulators means, different phytohormones could be kept over there, some nutrients could be given over there, which will serve as a source of nutrition during the transfer process or during the initial growth of the somatic embryo. Ok! So, the supplementation improves the germination efficiency and overall viability of the encapsulated somatic embryo.

As a result of these improvements, the synthetic seeds demonstrate enhanced resilience during storage, means, if nutrient is there definitely, they can sustain for longer period of time, they can maintain viability for an extended period including up to 6 months particularly when stored at a temperature of around 40 ^{o}C. So, now we will be discussing the addition of adjuvants to the matrix to safeguard the embryo from desiccation and potential physical harm, various beneficial substance such as nutrients, fungicides, pesticides, antibiotics and microorganisms like *Rhizobia* can be included within the encapsulation matrix also. So, in addition, the inclusion of activated charcoal serves to enhance the development of vitality of the encapsulated somatic embryos. This is not only is in efficient transformation of embryos, but also helps in retaining nutrients within the hydrogel capsules. So, the activated charcoal application was found to be highly beneficial.

Moreover, the activated charcoal facilitates gradual release of these nutrients to the growing embryo, ensuring a sustained and optimal nourishment for healthy growth and

development. Ok! So, gradual release of the nutrients could be maintained using activated charcoal. So, these are the references of this particular part. Thank you.