Brief content of lectures on discipline "**Basics of biotechnology"**

Lecture 6. Introduction. The aim of Plant biotechnology. Basic direction in Plant biotechnology. Cell technologies for receiving important products derived from plant material

L. 1 **Plants biotechnology**

History of Plant biotechnology

Hybrid breeding

Modern Plant breeding

Why plant biotechnology?

The word "biotechnology" was first used in 1917 to describe

processes using living organisms to make a product or run a process, such as industrial fermentations. Biotechnology began when humans started to plant their own crops, domesticate animals, ferment juice into wine, make cheese, and leaven bread.

Present definition of biotechnology "Any technological application that uses biological systems, living organisms, or derivatives theory, to make or modify

products or processes for specific use''

Plant biotechnology

"Plant biotechnology describes a precise process in which scientific techniques are used to develop useful and beneficial plants''

The field of plant biotechnology is concerned with developing ways to improve the production of plants in order to supply the world's needs for food, fiber and fuel.

In addition, plants provide us with many pharmaceuticals and industrial compounds.

As our population grows, our needs also grow.

To increase the quantity of crop production as well as to produce specific characteristics in plants, biotechnologists are using selective gene techniques.

History of Plant biotechnology

Early Plant breeding

Humans domesticate crops.

Breed plants to further improve desirable characteristics.

Plant breeding 12,000 years ago

Traditional plant breeding selects mutants for best yield and quality (e.g., tomatoes).

Classical Plant breeding.

Cross-breeding to strengthen traits.

Charles Darwin publishes the theory of evolution by natural selection. The founding of the science of genetics.

Gregor Mendel discovers the laws of inheritance by studying flowers in his garden. The science of genetic begins.

Hybrid breeding

Two parental lines of normally outbreeding species are inbred through several self-pollinations.

When crossing such lines the first generation has hybrid vigour.

The vigour gradually disappears over the next generations so new sowing seeds have to be purchased every year .

Selection operates on desirable traits, not on survival in the wild.

Modern Plant breeding

A basic type of modern plant breeding:

Mutation breeding

Green revolution

Plant tissue culture breeding

Mutation breeding

Seeds are treated with

either radiation or

mutagenic chemicals to induce larger or smaller

lesions in the genes.

The mutations are at random over the genome.

Usually mutation results

in a loss of function of

Genes.

Green revolution (1960-1970)

Green revolution' leads to greatly increased crop yields based on the incorporation of **dwarfing genes** discovered by Norman Borlaug and the widespread use of agrochemicals.

Plant tissue culture breeding

The process of selectively mating plants in aseptic culture.

Embryo rescue

Somaclonal variation selection

Somatic hybrid (i.e. fusion protoplast).

Generation of haploid (i.e. anther/microspore culture)

Highlights of Plant tissue culture

1902. Gottlieb Haberlandt proposed that all cells are totipotent. Totipotent.

Totipotency (Lat. *totipotentia*, "ability for all [things]") is the ability of a single cell to divide and produce all of the differentiated cells in an **organism.**

Spores and **zygotes** are examples of totipotent cells.

In the spectrum of cell potency, totipotency represents the cell with the greatest **differentiation** potential, being able to differentiate into any **embryonic** cell, as well as extraembryonic cells. In contrast, pluripotent cells can only differentiate into embryonic cells

History of Plant biotechnology

1904

Hanning isolated nearly mature zygotic embryos from seeds of Crucifers and successfully grew them to maturity in a defined medium.

1925

Laibach isolated and grew embryos of interspecific cross *Linum perenne* and *L. austriacum* that aborted in vivo

1948

Folke Skoog discovered that kinetin could induce organogenesis in callus culture of tobacco.

1957.

Skoog and Miller demonstrated the effects and interaction of phytohormones

Auxin : cytokin > 1 root formation

Auxin : cytokin <1 shoot formation

Auxin : cytokin = 1 callus formation

History of Plant biotechnology

1964

Haploid plants derived from cultured Datura anthers

1972

First interspecific hybridization of Nicotiana sp. by fusing protoplast

1974

Haploid plants derived from cultured tobacco microspores

1977

Successful integration of T-DNA in plants.

Two major areas of plant biotechnology:

Plant Tissue Culture (plants cloning)

Recombinant DNA technology (gene cloning)

Why plant biotechnology?

Human population is rapidly outgrowing.

Worlds' current status

For higher yield

Conventional Breeding

Ideotype Breeding

Hybrid Breeding

Wide Hybridization

Mutation Breeding

Germplasm Breeding

The scope of plant biotechnology

Plant genetic engineering

Plants micropropagation

Plant mutation cloning

Plant cells technology

Impact of plant biotechnology

More crop

More money

Environmental impact

In vitro conservation

Pesticide reduction

Health impact Society impact

More food

Better food

Society impact

Plant Tissue Culture (PTC):

Plant tissue culture is the sterile, in vitro cultivation of plant parts. Plants have the ability for differentiated cells revert to an undifferentiated state called callus.

These cells will then divide and then differentiate back to somatic embryo cells that will regenerate the entire plant.

Plant Tissue Culture (PTC):

Plants cultured in vitro yield thousands of genetically identical plants (clones) from a single plant.

This process is called micropropagation and is used to commercially propagate plants asexually. The rapid multiplication allows breeders and growers to introduce new cultivars much earlier than they could by using conventional propagation techniques, such as cuttings.

Plant Tissue Culture (PTC):

Through the use of biotechnology, desirable genetic traits can be transferred from one organism to another by transfer of DNA.

Many more plants with the desirable DNA can be regenerated from small pieces of the transformed plant tissue.

Examples of plants produced using tissue culture include the large variety of ornamental plants; agricultural crops such as strawberry, banana, potato, and tomato; and a variety of medicinal plants.

Plant Tissue Culture (PTC):

Commercial tissue culture involves exposing plant tissue to a specific regimen of nutrients, hormones, and light under sterile conditions to produce many new plants over a very short period of time.

LECTURE 7.

L. 7 Micropropagation technologies of plants.

What is Tissue Culture? Types,

Techniques and

Major Steps of Tissue Culture (Plants)

Process

Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition.

Plant tissue culture is widely used to produce clones of a plant in a method known **as micropropagation.**

Different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation, including

Plant tissue culture

The production of exact copies of plants that produce particularly good flowers, fruits, or have other desirable traits.

To quickly produce mature plants.

The production of multiples of plants in the absence of seeds or necessary pollinators to produce seeds.

The regeneration of whole plants from plant cells that have been genetically modified.

The production of plants in sterile containers that allows them to be moved with greatly reduced chances of transmitting diseases, pests, and pathogens.

The production of plants from seeds that otherwise have very low chances of germinating and growing, i.e.: orchids and *Nepenthes*.

To clear particular plants of viral and other infections and to quickly multiply these plants as 'cleaned stock' for horticulture and agriculture.

Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant (totipotency).

What is Tissue Culture? Types, Techniques and Process

In biological research, tissue culture refers to a method in which fragments of a tissue (plant or animal tissue) are introduced into a new, artificial environment.

In artificial environment fragments of a tissue continue to function or grow.

While fragments of a tissue are often used, it is important to note that entire organs are also used for tissue culture purposes.

The application of plant tissue cultures

in fundamental and applied studies on various biological species is rapidly growing.

The use of in vitro technology for commercial propagation of plant species and for the production of bioactive components from them has become profitable industry worldwide.

A whole plant can be regenerated from a small tissue or plant cells in a suitable culture medium under controlled environment.

The plantlets so produced are called tissue-culture raised plants.

These plantlets are a true copy of the mother plant and show characteristics identical to the mother plant.

For example, if the mother plant is a high yielding plant the plantlets will also be high yielding.

Many plant species are presently being propagated through tissue culture successfully.

The capacity of a single cell to grow into a complete plant is termed as Totipotency,

Plant tissue culture can be initiated from almost any part of a plant however, for micropropagation or direct shoot regeneration, meristemetic tissue such as shoot tip is ideal.

The physiological state of the plant has an influence on its response to tissue culture.

The mother plant must be healthy and free from obvious signs of disease or pest.

The shoot tip explants being juvenile contain a higher proportion of actively dividing cells. It is important to use quality mother plant stock to initiate cultures.

Tissue culture is the *in vitro* aseptic culture of cells, tissues, organs or whole plant under controlled nutritional and environmental conditions often to produce the clones of plants.

The resultant clones are true-to type of the selected genotype.

The controlled conditions provide the culture an environment conducive for their gro**wth and multiplication.**

These conditions include:

proper supply of nutrients,

pH medium,

adequate temperature and

proper gaseous and liquid environment.

Types of Tissue Culture.

Seed Culture

Seed culture is the type of tissue culture that is primarily used for plants such as orchids.

For this method, explants (tissue from the plant) are obtained from an in-vitro derived plant and introduced in to an artificial environment, where they get to proliferate.

In the event that a plant material is used directly for this process, then it has to be sterilized to prevent tissue damage and ensure optimum regeneration.

Embryo Culture

Embryo culture is the type of tissue culture that involves the isolation of an embryo from a given organism for in vitro growth.

*Note, the term embryo culture is used to refer to sexually produced zygotic embryo culture.

Embryo culture may involve the use of a mature of immature embryo. Whereas mature embryos for culture are essentially obtained from ripe seeds, immature embryo (embryo rescue) involves the use of immature embryos from unripe/hybrid seeds that failed to germinate. In doing so, the embryo is ultimately able to produce a viable plant

Embryo Culture

For embryo culture, the ovule, seed or fruit from which the embryo is to be obtained is sterilized, and therefore the embryo does not have to be sterilized again.

Salt sucrose may be used to provide the embryo with nutrients.

The culture is enriched with organic or inorganic compounds, inorganic salts as well as growth regulators.

Embryo culture

is a type of plant tissue culture that is used to grow embryos from seeds and ovules in a nutrient medium.

In embryo culture, the plant develops directly from the embryo or indirectly through the formation of callus and then subsequent formation of shoots and roots.

The technique is developed to break seed dormancy, test the vitality of seeds, production of rare species and haploid plants.

It is an effective technique that is employed to shorten the breeding cycle of plants by growing excised embryos and results in the reduction of long dormancy period of seeds.

Embryo Culture

Intra-varietal hybrids of an economically important energy plant "Jatropha" have been produced successfully with the specific objective of mass multiplication.

Somatic embryogenesis and plant regeneration has been carried out in embryo cultures of Jucara Palm for rapid cloning and improvement of selected individuals.

In addition, conservation of endangered species can also be attained by practicing embryo culture technique. Recently a successful protocol has been developed for the *in vitro* propagation of *Khayagrandifoliola* by excising embryos from mature seeds.

The plant has a high economic value for timber wood and for medicinal purposes as well. This technique has an important application in forestry by offering a mean of propagation of elite individuals where the selection and improvement of natural population is difficult.

Callus Culture

Callus - This is the term used to refer to unspecialized, unorganized and a dividing mass of cells. A callus is produced when explants (cells) are cultured in an appropriate medium - A good example of this is the tumor tissue that grows out of the wounds of differentiated tissues/organs.

In practice, callus culture involves the growth of a callus (composed of differentiated and non- differentiated cells), which is the followed by a procedure that induces organ differentiation.

Callus Culture

For this type of tissue culture, the culture is often sustained on a gel medium, which is composed **of agar and a mixture of given macro and micronutrients** depending on the type of cells.

Different types of basal salt mixtures such as murashige and skoog medium are also used in addition to vitamins to enhance growth.

Organ Culture

Organ culture is a type of tissue culture that involves isolating an organ for in vitro growth.

Any organ plant can be used as an explant for the culture process (Shoot, root, leaf, and flower).

Organ Culture

With organ culture, or as is with their various tissue components, the method is used for preserve their structure or functions, which allows the organ to still resemble and retain the characteristics they would have in vivo.

New growth (differentiated structures) continues given that the organ retains its physiological features.

As such, an organ helps provide information on patterns of growth, differentiation as well as development.

There are number of methods that can be used for organ culture

These include;

Plasma clot method - The method involves the use of a clot that is composed of plasma and chick embryo extract (or any other extract) in a watch glass.

This method is particularly used for the purposes of studying morphogenesis in embryonic organ rudiments and more recently for studying the actions of various hormones, vitamins and carcinogens of adult mammalian tissues.

There are number of methods that can be used for organ culture. These include;

Raft method –

For this method, the explant is placed on a raft of lens paper/rayon acetate and floated on a serum in a watch glass.

Method of organ culture

Agar gel method –

The medium used for this method is composed of a salt solution, serum as well as the embryo extract or a mixture of various amino acids and vitamin with 1 percent agar.

The explant has to be subcultured every 5 to 7 days.

The method is largely used for the study of developmental aspects of normal organs and tumors.

Method of organ culture

Grid method –

Grid method, as the name suggests involves the use of perforated stainless steel sheet, on which the tissue of interest is placed before being placed in a culture chamber containing fluid medium.

LECTURE 8. Cell engineering of plants. Cell selection

Cell selection

The process of selecting cells exhibiting specific traits within a group of genetically different cells. Selected cells are often sub-cultured onto fresh medium for continued selection and exposed to an increased level of the selection agent to eliminate false positives.

Tissue culture and engineering

Cell culture is a fundamental component of tissue culture and tissue engineering, as it establishes the basics of growing and maintaining cells *in vitro*. The major application of human cell culture is in stem cell industry, where mesenchymal stem cells can be cultured and cryopreserved for future use. Tissue engineering potentially offers dramatic improvements in low cost medical care for hundreds of thousands of patients annuall

Vaccines

Plant cell culture methods

Plant cell cultures are typically grown as cell suspension cultures in a liquid medium or as callus cultures on a solid medium. The culturing of undifferentiated plant cells and calli requires the proper balance of the plant growth hormones auxin and cytokinin.

The culture of viruses requires the culture of cells of mammalian, plant, fungal or bacterial origin as hosts for the growth and replication of the virus. Whole wild type viruses, recombinant viruses or viral products may be generated in cell types other than their natural hosts under the right conditions. Depending on the species of the virus, infection and viral replication may result in host cell lysis and formation of a viral plaque.

Applications of cell culture]

Mass culture of animal cell lines is fundamental to the manufacture of viral vaccines and other products of biotechnology. Culture of human stem cells is used to expand the number of cells and differentiate the cells into various somatic cell types for transplantation.[26] Stem cell culture is also used to harvest the molecules and exosomes that the stem cells release for the purposes of therapeutic development.

Biological products produced by recombinant DNA(rDNA) technology in animal cell cultures include enzymes, synthetic hormones, immunobiologicals (monoclonal antibodies, interleukins, lymphokines), and anticancer agents. Although many simpler proteins can be produced using rDNA in bacterial cultures, more complex proteins that are glycosylated (carbohydrate-modified) currently must be made in animal cells.

An important example of such a complex protein is the hormone erythropoietin. The cost of growing mammalian cell cultures is high, so research is underway to produce such complex proteins in insect cells or in higher plants, use of single embryonic cell and somatic embryos as a source for

direct gene transfer via particle bombardment, transit gene expression and confocal microscopy observation is one of its applications. It also offers to confirm single cell origin of somatic embryos and the asymmetry of the first cell division, which starts the process.

Cell culture is also a key technique for cellular agriculture, which aims to provide both new products and new ways of producing existing agricultural products like milk, (cultured) meat, fragrances, and rhino horn from cells and microorganisms. It is therefore considered one means of achieving animal-free agriculture. It is also a central tool for teaching cell biology.

Cell culture in two dimensions

Research in tissue engineering, stem cells and molecular biology primarily involves cultures of cells on flat plastic dishes. This technique is known as two-dimensional (2D) cell culture, and was first developed by Wilhelm Roux who, in 1885, removed a portion of the medullary plate of an embryonic chicken and maintained it in warm saline for several days on a flat glass plate. From the advance of polymer technology arose today's standard plastic dish for 2D cell culture, commonly known as the Petri dish. Julius Richard Petri, a German bacteriologist, is generally credited with this invention while working as an assistant to Robert Koch. Various researchers today also utilize culturing laboratory flasks, conicals, and even disposable bags like those used in single-use bioreactors.

Aside from Petri dishes, scientists have long been growing cells within biologically derived matrices such as collagen or fibrin, and more recently, on synthetic hydrogels such as polyacrylamide or PEG. They do this in order to elicit phenotypes that are not expressed on conventionally rigid substrates. There is growing interest in controlling matrix stiffness,

a concept that has led to discoveries in fields such as:

- Stem cell self-renewal.
- Lineage specification.
- Cancer cell phenotype.
- Fibrosis.
- Hepatocyte function. Mechanosensing

Engineering the plant cell factory for secondary metabolite production.

Plant secondary metabolism is very important for traits such as flower color, flavor of food, and resistance against pests and diseases. Moreover, it is the source of many fine chemicals such as drugs, dyes, flavors, and fragrances. It is thus of interest to be able to engineer the secondary metabolite production of the plant cell factory, e.g. to produce more of a fine chemical, to produce less of a toxic compound, or even to make new compounds, Engineering of plant secondary metabolism

is feasible nowadays, but it requires knowledge of the biosynthetic pathways involved. To increase secondary metabolite production different strategies can be followed, such as overcoming rate limiting steps, reducing flux through competitive pathways, reducing catabolism and overexpression of regulatory genes. For this purpose genes of plant origin can be overexpressed, but also microbial genes have been used successfully. Overexpression of plant genes in microorganisms is another approach, which might be of interest for bioconversion of readily available precursors into valuable fine chemicals. Several examples will be given to illustrate these various approaches. The constraints of metabolic engineering of the plant cell factory will also be discussed. Our limited knowledge of secondary metabolite pathways and the genes involved is one of the main bottlenecks.

LECTURE 9. **Selection of Somatic Hybrids: 6 Strategies | Biotechnology**

Somatic hybridization is an important tool of plant breeding and crop improvement by the production of interspecific and intergeneric hybrids.

The following points highlight the six main screening strategies that are to be enforced for the selection of ideal somatic hybrids. The strategies are: 1. Microscopic Visual Selection 2. Auxin Autonomy 3. Chlorophyll Complementation 4. Biochemical Selection 5. Verification by Molecular Screening 6. Chromosomal Analysis.

Strategy # 1. Microscopic Visual Selection:

It is based on the fusion between coloured and colourless protoplast. Microscopic observation of heterokaryons, formed due to complete integration of structural characters of both parental protoplasts and subsequent culture under non-selection conditions and the development of heterokaryons facilitates the selection of potential hybrid cell line.

Strategy # 2. Auxin Autonomy:

In this selection process, protoplasts are subjected to screening by its potential to grow into cell on the medium devoid of auxin. Fusion of protoplasts between the same genotype and unfused protoplast fails to grow in absence of auxin in the medium.

However, fusion between the two target genotype potentiates to grow on the medium in absence of auxin. Mixture of two genetic materials allows hybrid cell lines to become auxin autonomy. Selection of the hybrids based on these approaches has been successful in certain members of leguminaceae.

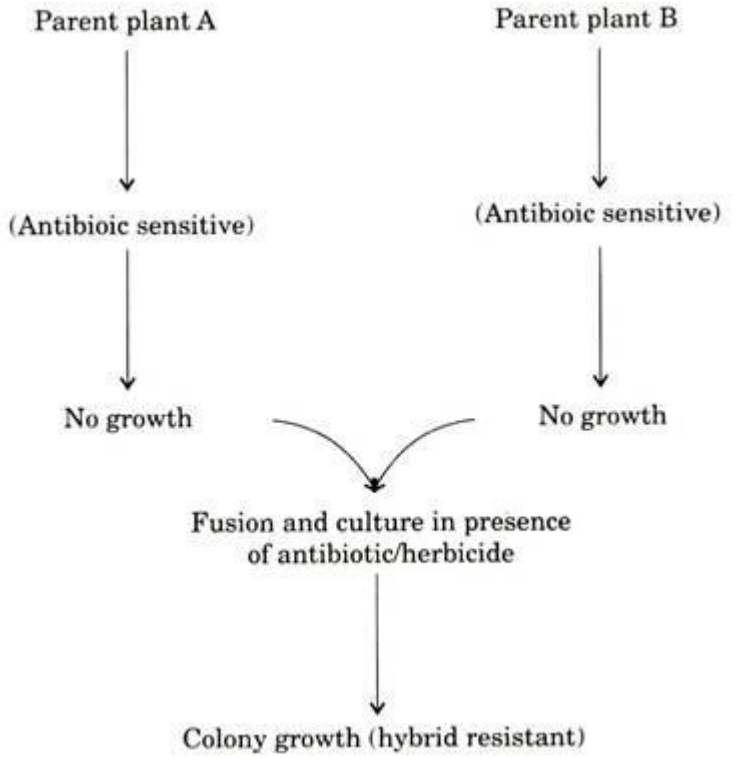
Strategy # 3. Chlorophyll Complementation:

This approach has been successfully implicated in the selection of somatic hybrids in light sensitive tobacco varieties. Development of green colour colony in culture medium ensures hybrids. This was accomplished by fusion between two homozygous recessive albino mutants of tobacco.

trategy # 4. Biochemical Selection:

This is based on conferring resistance due to dominant character against certain chemicals like antibiotics, herbicides, etc. These are being considered as resistant markers in the selection of somatic hybrids. For example, protoplast obtained from each parent, and grown separately in the medium contains antibiotics or herbicides, each parental line exhibiting sensitivity.

owever, protoplast fusion between two parental types when cultured in the medium containing these chemicals exhibit resistance. The sensitivity trait of each parent will be dominated by resistant trait and will grow on the medium containing antibiotics or herbicides.



soenzymes are multiple molecular forms of the same enzyme and execute the same function. Depending on the genotype, isoenzyme acts as specific blue print and exhibit specific banding pattern with respect to their complementation of each parental type. In biochemical analysis, electrophoretic banding of isoenzyme can be analyzed for the verification of hybridity.

Different nature of protoplasts (fused, unfused) are subjected for electrophoretic separation of isoenzyme bands on acrylamide gel. Somtic hybrids display characteristic banding pattern of both the

parents. Comparative enzyme profiles of each parental line and hybrid can be seen. The isoenzymes, which have been extensively used in biochemical analyses, are esterases, peroxidases, amylases and alcohol dehydrogenases.

Strategy # 5. Verification by Molecular Screening:

Several molecular techniques like RAPD, RFLP and availability of microsatellites are employed in the screening as well as verification of specific somatic hybrids. Restriction digestion of DNA obtained from unfused and fused protoplast exhibit specific banding profiles and ensures confirmation of hybrids.

Restriction digestion of organelle DNA can boost up effective screening process of hybrid lines and verification of somatic hybrid plant in germplasm. Verification of somatic hybrid, Nicotiana glauca, was successfully carried out by assessing restriction fragments of nuclear DNA, which encodes ribosomal RNA. Recently, availability of specific primers for somatic hybrid has been utilized for hybrid identification through PCR technology.

Strategy # 6. Chromosomal Analysis:

Chromosome count can be adapted for the identification of somatic hybrid cell lines. Somatic hybrid contains sum of chromosomes in the protoplst of two parental types. Besides, variation in chromosome number is common in hybrids. Genetic variation due to structural alteration in chromosome might help in the identification of hybrids.

Polyploid conditions have been witnessed in the protoplast culture, which involves the production of inter specific and inter generic somatic hybrids. On several occasions, variation in the chromosome number is mainly due to multiple fusion of protoplast. In addition, unequal rate of DNA replication in the hybridoma cells results in asymmetric hybrids and consequently exhibit chromosomal variations.

LECTURE 10. Methods of Fertilization in vitro. Haploid technology

What is a haploid plant?

Haploid plants originate from gametes (or gamete-like cells) that do not go through fertilization, but can still generate a viable individual. Therefore, haploids contain only the chromosome set found after meiosis in male (sperm cells) or female (egg cells) gametes. This chromosome set ‘n’ corresponds to only half of the chromosome set found in the fertilization product (zygote) and other somatic cells. Depending on whether the single set of chromosomes comes from the maternal or paternal side, the plant is referred to as maternal haploid and paternal haploid, respectively.

What is a doubled haploid (DH) plant? In a DH plant, the chromosome set of a haploid plant has been doubled spontaneously or artificially.

Chromosome doubling is necessary since haploid plants are generally frail, have reduced organ size and are not fertile. The most commonly used chemical agent to render haploid plantlets diploid is colchicine, which blocks cell division without blocking chromosome Quick guide duplication. This treatment acts like a 'copy–paste' of the haploid genome into a diploid genome. Consequently, in DH plants all loci are homozygous. Chromosome doubling creates 'pure' homozygotes or fully inbred lines (Figure 1). Why is doubled haploid technology impactful for agriculture? Doubled haploid technology comprises both the production of haploid plants and the chromosome doubling process (Figure 1). It has become an important tool in plant breeding, since it shortens the time needed to create pure homozygous lines, which can either be released directly to farmers as cultivars or used as genitors (inbred lines) for the production of hybrid seeds. The primary advantage of DH plants is to possess a phenotypic stability due to the fact that all alleles are in a homozygous state. In short, DH technology increases the effi ciency of plant breeding. What are the different methods to produce haploid plants? The numerous methods to obtain haploid plants can be classifi ed into two categories (Figure 1). Firstly, in vitro methods are based on the culture of haploid cells and their differentiation into haploid embryos and ultimately haploid plants. Both male (microspores or pollen) and female haploid cells (megaspores or ovules) are used, depending on the responsiveness of the cells in a given species. Secondly, in situ methods make use of particular pollination techniques using irradiated pollen, inter-specifi c crosses or so-called 'inducer lines'. What is a haploid inducer line? Haploid inducer lines are routinely used in plant breeding for maize only, and thus represent an exception. Maize haploid inducer lines all derive from a particular genotype discovered in the 1950s that possesses the ability to induce the development of haploid embryos on a maize line of interest upon pollination with the inducer pollen. The pollen from the inducer line triggers the development of the egg cell into an embryo containing only a haploid maternal genome. This process is called in vivo gynogenesis (Figure 2). Recently, haploid inducer lines have also been created in Arabidopsis thaliana, Brassica juncea and maize by the use of engineered centromeric histone 3 (CENH3) variants. However, this haploid induction method has not been reported in plant breeding programs so far. How does in situ haploid induction work in maize? All fl owering plants are characterized by a particular way of sexual reproduction called double fertilization. It consists of two parallel fusion events between male and female gametes (Figure 2). The haploid egg cell is fertilized by one haploid male gamete and becomes the diploid embryo. At the same time, the diploid nucleus of the central cell is fertilized by the second haploid male gamete of the same pollen tube to form a seed nutritive tissue, the triploid endosperm (Figure 2). Pollination by a maize inducer line results in an atypical fertilization event in which only the

central cell is fertilized normally by a male gamete, and the egg cell develops into a haploid embryo lacking the paternal genome (Figure 2). Note that after pollination by a maize inducer line, only about 10% of the developing seeds contain a haploid embryo, the remaining 90% are normal diploid embryos. What are the molecular players behind in situ haploid induction in maize? The inducing capacity of inducer lines has been recently tracked to a 4 bp insertion at the end of the coding sequence of a gene named ZmPHOSPHOLIPASE A1 (ZmPLA1). NLD/MTL/ZmPLA1 is specifi cally expressed in male gametes and encodes a patatin-like phospholipase A localized at the plasma membrane of the male germ unit. The predicted truncated protein is not detectable in inducer lines and loses its plasma membrane anchorage in a heterologous system. How the biochemical function of NLD/MTL/ ZmPLA1 relates to its inducing capacity is still unresolved, and either a structural or a signaling function has been hypothesized. Whereas loss of NLD/MTL/ZmPLA1 is suffi cient to trigger haploid induction, quantitative trait locus (QTL) analysis demonstrated that additional, currently unknown players take part in the process and influence the effi ciency of haploid induction. What future improvements are needed for DH technology? In plant breeding, and apart from maize, the production of DH plants requires at least an in vitro-based process (Figure 1), the success of which remains highly species- and genotype-dependent, as well as labor-intensive and time-consuming.

The DH technology has not yet been applied to all breeding programs, and there are still some major crop species (e.g., soybean, tomato, sunfl ower) that are recalcitrant to the currently available procedures, or for which present protocols are not effi cient enough. The understanding of the maize in situ system should help to extend the DH technology to more species or breeding programs by either directly knocking out the functional ortholog of the maize gene, or if needed by transferring the cellular and molecular knowledge acquired on maize. In addition to these applications, the identifi cation of the NLD/MTL/ZmPLA1 gene offers a unique opportunity to explore the many mysteries of double fertilization in plants.