Plant breeding and improvement

Plant tissue culture offers excellent potential for obtaining plants with special characteristics by changing the genetic makeup of the resulting individual. Despite the significant development witnessed in the fields of life sciences, there are still many problems facing plant breeders and scientists, such as the time factor and the problem of incompatibility between plants to conduct different crosses to transfer a specific trait from one plant to another. Therefore, researchers and plant breeders have used plant tissue culture to overcome problems since the last century. The most important technologies used in this field are the cultivation of stems and pollen, the cultivation of embryos and eggs, performing protoplasmic hybridization and inducing mutations in vitro, among others.

Anther and pollen culture

The sex cells in diploid plants carry the primary single number of chromosomes. In many cases, plant breeders wish to obtain plants with a single set of chromosomes and double their chromosome number to produce diploid plants that can be wholly pure and true breeding. Among the available methods are cultivating anthers and pollen and growing them directly into monochromosomal plants.

Anther development stage

The conversion of pollen taken from angiosperms from the standard form of development (germination and pollen tube formation) to the state of growth and expansion into plants only occurs when they are planted at the optimal stage of development. This stage of tetraploid formation is considered after the end of the mitotic phase and ends before starch deposition in the early gametophyte stage.

Depending on the internal structure of the stages of pollen development, pollen can be divided into several developmental stages depending on their age to compare the response of the seed to the process of cultivation.

Pollen grain culture

The importance of cultivating pollen grains after separating them from the anthers of haploid plants lies in the fact that developing the entire anther on certain nutrient media may lead to the formation of plants emerging from pollen grains or sometimes from anther tissues that are usually genetically heterogeneous. To overcome such obstacles, it is preferable to cultivate Mature or young pollen grains after being separated from the anther. Using a glass homogenizer, pollen grains from sterilized mycelium are usually preferred in a liquid nutrient medium. The pollen grains are separated from the liquid nutrient medium using centrifugation at a relatively slow speed and then placed on a solid medium specified for this purpose. The mature pollen grains of many gymnosperms form callus when grown on solid nutrient media. In contrast, the pollen grains of angiosperm plants usually do not form callus when grown on suitable nutrient media.

Androgenesis is the pollen development and passage process through a series of cell divisions and differentiation based on its developmental energy to form a single-chromosomal plant. There are two types of Androgenesis process:

1. Direct androgenesis: In which the pollen grain behaves like a fertilized egg (Zygot), undergoing a series of changes to form a somatic embryo. This embryo grows to give a plant with a single set of chromosomes, as in the Datura and tobacco plants.
2. Indirect androgenesis: In which the pollen grain repeatedly divides to form

callus tissue, which differentiates into plants with a single set of chromosomes through the formation of asexual (somatic) embryos or branches that are later rooted, as happens in wheat, barley, and rye plants.

Embryo Culture

Embryo miscarriage is the most common case during the hybridization process, whether between intergeneric crosses, varieties, or species of the same genus (Interspecific crosses). Although Fertilization has occurred, the embryo is aborted at certain stages of seed development due to the endosperm's decay and the embryo's deprivation of the necessary food. The embryo can be saved for its growth and development by isolating and cultivating it on a specific culture medium, then developing it into a seedling and a complete plant.

Embryo cultivation requirements

One of the most important factors determining the success of embryo transplantation is choosing the appropriate medium that provides the different needs of the embryo at various stages of its development. Adult embryos (fully formed in the torpedo stage) only need a solution of mineral salts and sucrose. In contrast, immature embryos (in the spherical stage) do not grow on this medium and need specific additives. Two main steps determine the development of embryos:

Heterotrophic Stage: The fetus depends on the endosperm and other surrounding tissues for its nutrition, growth, and development.

- Autotrophic stage: At this stage, the fetus can benefit from the nutrients and sugar in the nutritional environment to build the materials necessary for its growth and development, and thus, it is autotrophic. The fetus moves from the saprophytic (parasitic) stage to the autotrophic location as the stages of its development continue. It has been found that the fetus is parasitic. Nutrition in the shepherd's bag plant until the globular stage, then it becomes autotrophic in the later stages (the torpedo stage), and the needs of the fetus become less as it grows older.

Applications of Embryo Culture

1. Obtaining rare hybrids: This is the most common and common use of embryo culture. In many crosses between species or species, Fertilization occurs naturally, and the resulting embryo develops naturally. However, the lack of endosperm tissue development leads to the embryo's early death, making it impossible to obtain Germinable seeds. Researchers have indicated the possibility of developing embryos resulting from crossbreeding between different species or genera on artificial nutritional media, provided that the separation occurs at the appropriate time (before the miscarriage of the embryo). Therefore, this technique is used to cultivate or produce plant hybrids from immature crosses due to the obstacles that appear after Fertilization.
2. Shortening education and improvement cycles: Raising horticultural plants, especially trees and shrubs, requires an extended period due to the long dormancy period that the seeds go through, but thanks to the development of these embryos on artificial nutrient media, this period can be shortened. In wild apples, it was observed that the seeds begin to germinate 48 hours after they are planted. On artificial nutrient media, after three weeks, it was possible to obtain seedlings that could be transplanted, and after five months, the seedlings reached a length of 1 m, while the seeds planted in the soil required nine months to germinate. It was also noted that the hybrid plants of apricot and peach resulting from growing the embryos on nutrient media were better

than those produced from seeds regarding flowering enlargement and number of flowers per plant.

3. Accurate propagation of rare species, such as conifers and legumes, is difficult to germinate and has a meagre germination rate.
4. An effective tool for checking seed purity.
5. It is used to study the growth and development of embryos and embryogenesis in plants.

Overview Culture

It is the transplantation of unfertilized ovaries to obtain single-chromosomal plants from the egg cell or any single cells in the embryonic sac. The process is called Gynogenesis. The first to write about Gynogenesis was San Noem in 1976 AD. It is a barley plant. Generally, the ovaries are grown floating on nutrient media containing low concentrations of auxins and incubated in the dark. This cultivation helps stimulate the ovaries while they are transferred to the regeneration medium, a solid and container medium—higher auxin concentrations and incubated in light.

Limitation of ovary culture

1. The response rate of ovaries to tissue culture is low (1-5%), and the number of plants formed from one ovary is small (1-2) plants.
2. Only a few types of plants have successfully grown their ovaries ex vivo.

Advantages of ovaries culture

1. It is used in studies on fruits' appearance, physiology, stages of development, and nutritional needs.
2. They are used in the case of male sterility in different crops.
3. Used for Fertilization and In vitro pollination.
4. It is used in cases of Embryo rescue after Fertilization resulting from miscarriage.
5. To reduce the proportion of Albino plants.

Ovule culture

The egg culture method is used instead of ovary culture in cases where a miscarriage occurs very early (immediately after the fertilization process). As an alternative to embryo transplantation if it is not possible or difficult to remove them at this very early stage. Usually, the eggs prefer several days after Fertilization and before the egg divides. Another problem facing plant breeders is incompatibility, whether the pollination is self-pollination or cross-pollination, especially genetically distant species. Pollen grains may mature early, before the stigmas mature and are ready for pollination. The pollen tube does not grow on the flower's stigmas or is short in length, and there are some inhibitors to the growth of pollen grains on the stigmas at times. The only way to overcome these problems is to use in vitro pollination and Fertilization. In this technology, the eggs can be isolated and cultivated on limited nutrient media and then Pollinate with pollen from the desired plant. Then, obtain seeds that later grow into seedlings. This technology was used to obtain seeds of living plants such as petunia, which are naturally incompatible.

Isolation and culture of protoplast

Protoplast

They are the bare living cells (without the cell wall) of the plant from which the wall has been removed either mechanically or by the action of enzymes that digest the cell wall. As a result of removing the wall, the barrier between the protoplasm (living matter) and the surrounding external environment is the plasma membrane through which dissolved materials are transferred from the plant cell. And to her. The isolated protoplasts must be placed in a neutrally osmotic nutrient medium to protect them from damage. Separating protoplasts is not a new phenomenon, but it was isolated in the early twentieth century by mechanical methods to study protoplasmic flow and was then used in many fields.

Protoplast culture applications

1. Isolated protoplasts can be induced to fuse to obtain hybrid plants. Although this phenomenon has been applied to several species of plants, fusion may not occur in some plant species. This method is used in species from which it is challenging to obtain hybrid species.
2. Isolated protoplasts can incorporate foreign materials and introduce them into the cytoplasm, as in plasmids, nuclei, and other organelles.
3. Protoplasts growing in certain nutritional media are suitable for studying cell wall formation.
4. Isolated protoplasts can be studied as a single system from which cells and, thus, plants with special specifications can be obtained.

Producing plants free of diseases, pathogens and viruses

Most plants, especially those propagated vegetatively, are infected with one or more pathogens such as fungi, bacteria, viruses, mycoplasma, and others. Shalek plants, for example, are attacked by 62 types of mycoplasma and viruses, which makes it necessary to plant the mother plants used for vegetative propagation annually to keep the production healthy and disease-free. Apical meristem cultivation techniques have been used to rid many plant species of pathogens.

Apical meristem culture

This method is considered the most suitable for producing virus-free plants in many economically important crops, such as fruits such as strawberries, bananas, pineapples, and citrus fruits, vegetable crops such as sweet potatoes, garlic, and cauliflower, and ornamental plants such as cloves, orchids, dandelions, dahlias, geraniums, and some bulbs such as cladiolus, freesia, amaryllis, iris, and lily. The reasons why the apical meristem is the best plant part for producing virus-free plants are:

1. It can reproduce in plants faster than other plant tissues when grown on a suitable medium.
2. Plants produced from it are genetically similar to their mothers because the genetic makeup of meristematic cells is regular and stable.
3. The meristematic shoots of plants infected with viruses are either free of viruses or contain deficient concentrations.

The gradual distribution of viruses at the tip of the branch enabled Morel and Martin 1952 to produce the first virus-free dahlia plant from an infected plant. In 1977, Quak mentioned several reasons for the meristematic peak being free of viruses:

1. Viruses are transmitted in the plant body through the vascular tissue, which is absent in the meristem.
2. The lack or absence of plasmodesmata protoplasmic connections in the apical meristem between rapidly dividing meristematic cells and the movement of viruses through these connections is prolonged.
3. The high structural activities in actively dividing meristematic cells do not allow the virus to multiply.
4. Substances that inhibit the effectiveness of the virus in some plant species were

found to have the highest concentration in the plant's apical meristems.

5. High auxin levels in the growing apex may inhibit virus replication.