3. Modes of Reproduction

Can you recall?

Reproduction is the production of new individuals by sexual or asexual method/ mode. Mode of reproduction determines the genetic constitution of crop plants, i. e., whether the plants are normally homozygous or heterozygous. This, in turn, determines the goal of a breeding programme. If the crop plants are naturally homozygous, *e.g.*, as in self pollinators like wheat, a homozygous line would be desirable as a variety. But if the plants are heterozygous naturally, *e.g.*, as in cross-pollinators like maize, a heterozygous population has to be developed as a variety. Consequently, the breeding methods have to be vastly different for the two groups of crop plant. A knowledge of the modes of reproduction of crop plants is also important for making artificial hybrids. Production of hybrids between diverse and desirable parents is the basis for almost all the modern breeding programmes.

The modes of reproduction in crop plants may be broadly grouped into two categories sexual and asexual.

3.1 SEXUAL REPRODUCTION

3.1.1 Definition : Sexual reproduction means fusion of male and female gametes to form a zygote, which develops into an embryo.

In crop plants, male and female gametes are governed by specialized structure known as flower.

3.1.2 Flower : A flower usually consists of pedicel, calyx (sepals), corolla (petals), androecium and gynoecium (stamens and pistil). It is called as complete flower. A flower containing both stamens and pistil is a perfect or hermaphrodite or bisexual flower. If it contains stamens but not pistil it is known as staminate or male flower, while a pistilate or female flower contains pistil but not stamens. Staminate and pistilate flowers occur on the same plant but at different location in a monoecious species such as maize, colocasia, castor, coconut, banana and cucurbits etc. However, in dioecious species, staminate or male and pistilate or female flowers occur on different plants e.g. papaya, date palm and hemp.

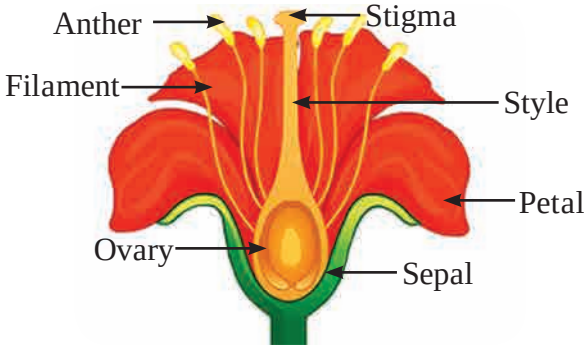


Fig. 3.1 Structure of a flower.

Can you recall?

Fertilization

The fusion of one of the two sperms with the egg cell producing diploid zygote is known as fertilization.

3.1.3 Significance of sexual reproduction :

Sexual reproduction makes it possible to combine genes from few or more parents into a single hybrid plant. Recombination among these genes produces a large number of different genotypes. This is an essential step in creating genetic variation through hybridization. Almost the entire plant breeding is based on sexual reproduction.

3.1.4 Advantages of sexual reproduction :

1. Sexual reproduction is simple and easy.
2. Plants produced long live; show greater tolerance to soil and climate.
3. This is the only way to reproduce where asexual means is not common.
4. Introduces variation into a population.
5. The species can adapt to new environment.
6. A disease is less likely to affect all the individuals in a population.

3.1.5 Disadvantages of sexual reproduction

1. Sexually propagated plants require more period for flowering.
2. Two parents are needed in **sexual reproduction**, and the offspring produced are genetically different from the parents.
3. Time and energy are needed to find a mate
4. Not possible for an isolated individual.

3.2 ASEXUAL REPRODUCTION

3.2.1 Definition : Asexual reproduction does not involve fusion of male and female gametes. New plants may develop from vegetative parts of the plant (*vegetative* reproduction) or seed may arise from embryos that develop without fertilization (apomixis).

3.2.2 Vegetative Reproduction : Reproduction by vegetative parts of plant. Types of vegetative reproduction is as follows.

1. Natural vegetative reproduction :

In nature, a new plant develops from a portion of the plant body. This may occur through modified underground and sub-aerial stems, and through bulbills.

a. Underground Stems :

The underground modifications of stem generally serve as storage organs and contain many buds. These buds develop into shoots and produce plants after rooting. Examples of such modifications are given in the figure.

Tuber: Potato.

Bulb : Onion, garlic.

Rhizome: Ginger, turmeric.

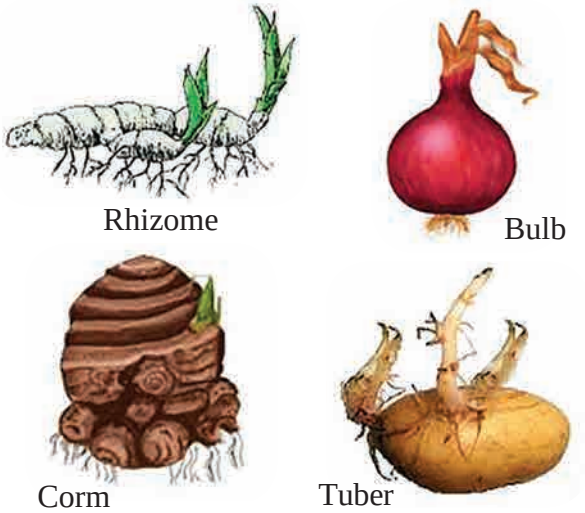


Fig. 3.2 Underground stem

b. Sub-aerial Stems :

These modifications include runner, stolon, sucker etc. Sub-aerial stems are used for the propagation of mint, date palm etc.

c. Bulbils :

Bulbils are modified flowers that develop into plants directly without formation of seeds. These are vegetative bodies; their development does not involve fertilization and seed formation. The lower flowers in the inflorescence of garlic naturally develop into bulbils. Scientists are trying to induce bulbil development in plantation crops by culturing young inflorescence on tissue culture media; it has been successfully done in cardamom.

Visit the field and study the natural vegetative reproduction in your area.

2. Artificial vegetative / Commercial reproduction :

It is commonly used for the propagation of many crop species, although it may not occur naturally in those species. Stem cuttings are commercially used for the propagation of sugarcane, grapes, roses, etc. Layering, budding, grafting and gootee are in common use for the propagation of fruit trees and ornamental shrubs. Techniques are available for vegetative multiplication through tissue culture in case of many plant species, and attempts are being made to develop the techniques for many others. In many of these species, sexual reproduction occurs naturally but for certain reasons vegetative reproduction is more desirable. Following are some important artificial reproduction practices.

a. Cuttings :

Many plants like rose, bougainvillea, croton, coleus, money plants and sugarcane etc., are grown through their stem cuttings. (Fig. 3.2 a) Cuttings of these plants can be grown even in water where they strike roots and develop adventitious buds.

Fig. 3.2(a) Cuttings for reproduction

Internet my friend

Obtain information regarding following types of cutting.
1. Stem cutting : Soft wood, semi-hardwood and hardwood cutting, 2. Leaf cutting, 3. Leaf bud cutting, 4. Root cutting

b. Simple tongue layering :

In this method, a lower branch of a plant is bent down and covered with moist soil leaving the growing tip above the soil. A slanting vertical cut is given on selected pencil size stems in the soil before it is bent down (Fig. 3.2b). In a few weeks time when enough roots have developed on the underground portion above the cut part, it is cut off from the parent plant and grown separately as an independent plant. Example: Guava etc.

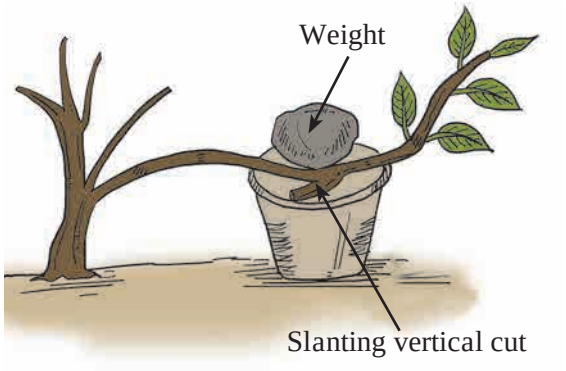


Fig. 3.2(b) Simple tongue layering

c. Air layering or *Gootee* :

It is a similar practice where bending of branches not possible because of the height of plant or due to woody nature of stem. In this method a ring of bark measuring 2-3 cms length is removed from a selected pencil size stem and it is covered with moist sphagnum moss and enclosed with a polythene sheet tightly tied with jute string. When roots appear, the stem is cut below the roots and planted to form a new plant.

Example : Pomegranate, Jasmin, Thuja etc.

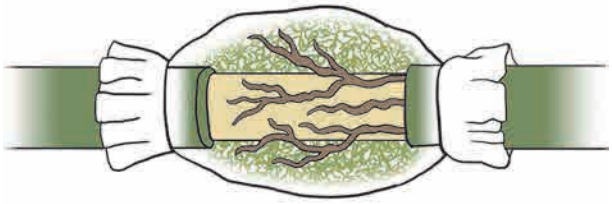


Fig. 3.2(c) Air layering

Try this

Collect information regarding other types of layering such as tip layering, simple tip layering, trench layering, serpentine layering and mound or stool layering.

d. Grafting :

It is especially important for propagation of seedless varieties of plants. It consists of inserting a small branch into a rooted plant. The rooted plant taken as a stock is resistant to disease and is physically sturdy. In this stock a branch is inserted which is known as scion. This scion is the stem cutting from the desired plant. Usually the grafted end of stock and scion fit well with each other and are bound firmly with tape or rubber-band until their tissues unite and vascular continuity is established. Grafting is mostly practiced in dicot plants. Grafting has been found extremely useful in propagating improved varieties of various flowers and fruits like rose, bougainvillea, citrus, mango, *chiku*, etc. The resultant grafted plants are dwarf in nature.

Do this

Searh information regarding following types of grafting :
A. Scion attached methods
B. Scion detached methods
C. Grafting on established trees
D. Methods of rejuvenation

e. Budding

Budding, often called bud grafting, is an artificial method of asexual or vegetative propagation in plants. Like grafting, this

method is employed to convert one plant (the rootstock) into another plant type with desirable characteristics. The bud of desirable plant is removed and inserted into the vertical slit opened on the stem of the rootstock. The resulting plant, in general, have dwarf stature and early maturity as compared to plants propagated from seed. e.g. Rose, Ber, etc.

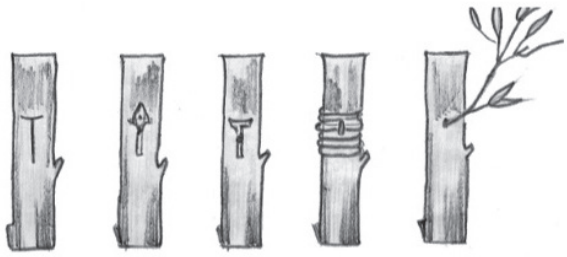


Fig. 3.2(d) T or shield budding

Internet my friend

Obtain information regarding following types of budding
A. T bdding or shield budding
B. Patch budding
C. Forkert budding
D. Flute budding
E. Ring budding
F. Chip budding

Try this

Practice any one of the artificial vegetative reproduction in near by plant nursery.

A. Advantages of vegetative reproduction :

Vegetative reproduction has several advantages. The main advantages are:

1. It is useful for obtaining large number of genetically identical individuals of genotypes , irrespective of the degree of heterozygosity.
2. Promising individuals occurring at any stage in a breeding programme can be easily picked up and maintained by asexual reproduction.
3. It makes use of desirable bud mutations. Mutants can be directly released as varieties.
4. (a) Rapid reproduction and spread. The desired varieties can thus be preserved genetically for further use. (b) Improved varieties of ornamental plants and fruit trees can be multiplied easily.
5. Vegetative propagation is a quicker, easier and a less expensive method of multiplying the plants.
6. Plants developed by this method are smaller in stature and hence harvesting become easy.
7. Using this method noble plants can be created.

B. Disadvantages of vegetative reproduction

1. It requires skilled manpower.
2. It is expensive. Plants have short life.
3. New varieties cannot be produced by this method except by mutation.
4. Diseases of the typical species are rapidly transmitted and can destroy a crop.
5. There is no genetic variation so, plants are less adaptable to enviroment.

3.2.3 Apomixis :

Apomixis refers to the development of seed without sexual fusion (Fertilization). In apomixis, embryo develops without fertilization. Thus, apomixis is an asexual means of reproduction. Apomixis is found in many crop species. Reproduction in some species occur only by apomixis. This apomixis is termed as obligate apomixis. But, in some species sexual reproduction also occurs in addition to apomixis. Such apomixis is known as facultative apomixis. There are four types of apomixis : *viz;*

1. Parthenogenesis
2. Apogamy
3. Apospory
4. Adventative embryony.

1. Parthenogenesis :

Parthenogenesis refers to development of embryo from the egg cell without fertilization. It is of two types 1. Haploid and 2. Diploid.

When the embryo develops from a haploid egg cell, it is known as haploid parthenogenesis. The plants which develop from such embryos are haploid and sterile. Haploid parthenogenesis is found in *Solanum nigrum*. Some times embryosac developes without reduction division. Such embryosac and all cells within it are diploid. It gives rise to diploid embryos. Such parthenogenesis is known as diploid parthenogenesis and has been reported in grasses like *Taraxacum*. In plant species like tobacco and rice, pollen grains may be induced to develop in embryos. This development of embryos from pollens of anthers is termed as androgenesis.

There are several causes of parthenogenesis
The main causes include 1. Inability of the pollen tube to discharge the contents inside the embryo sac 2. Insufficient attraction between male and female gametes 3. Early degeneration of the sperm 4. Very long style 5. Short pollen tube 6. Slow rate of pollen tube growth 7. Stimulation of pollen in the absence of pollen tube and 8. Incompatibility

2. Apogamy :

The origin of embryo from either synergids or antipodal cells of the embryosac is called apogamy. It is of two types viz, 1) haploid apogamy and 2) diploid apogamy. The synergids or antipodal cells may be haploid or diploid. If embryo develops from haploid synergids or antipodal cells, it is known as haploid apogamy. When the embryo develops from diploid synergids or antipodal cells, it is called as diploid apogamy.

3. Apospory :

In apospory first diploid cell of ovule lying outside the embryosac develops into another embryosac without reduction. The embryo then develops directly from the diploid egg cell without fertilization. Apospory is of two types viz., 1) generative apospory and 2) somatic apospory.

4. Adventive embryony :

The development of embryo directly from the diploid cells of ovule outside the embryosac belonging to either nucellus or integuments is referred to as adventive embryony. There is no production of another embryosac like apospory. This is a type of sporophytic budding which is very common in citrus and mango.

3.3 TISSUE CULTURE

Can you recall?

Totipotency is the inherent potentiality of a plant cell to give rise to a whole plant.

This is a capacity which is retained even after a cell has undergone final differentiation in the plant body. In plants, even highly mature and differentiated cells retain the ability to regenerate to a meristematic state as long as they have an intact membrane system and a viable nucleus. This is contradicting to animals, where differentiation is generally irreversible.

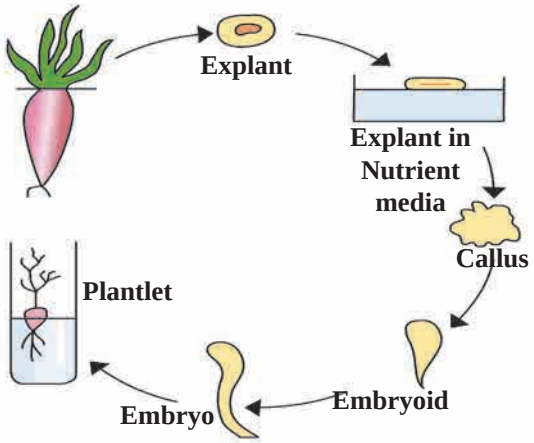


Fig. 3.3 Tissue culture technique (Totipotency)

3.3.1 Tissue Culture Technology :

Tissue culture technology is based on the theory of totipotency i.e. the ability of a single cell to develop into whole plant. The major components of the technology include choice of explant (excised part of plant), growing of explant on a defined medium in glass vessel (*in vitro*), elimination and or prevention of diseases, providing appropriate cultural environment and transfer of plantlets from glass vessel to natural environment (hardening). All these constitutes the protocol for tissue culture. It varies from species to species and variety to variety within the same species. However, it can be standardized through trial and error and ultimately it should be repeatable and reliable.

Plant tissue culture or micropropagation technology has made invaluable contribution to agriculture by enabling the production of disease free, quality planting material of commercial plants and fruit trees, throughout the year.

3.3.2 Definition :

Plant tissue culture is the technique of growing plant cells, tissues and organs in an artificially prepared nutrient medium, under aseptic and controlled conditions.

Tissue culture involves production of plants from very small plant parts, tissues or cells grown aspetically in a test tube or other suitable container where the environment and nutrition can be rigidly controlled.

Plant tissue culture as such is not a separate branch of plant science, but it is actually a collection of experimental methods or techniques. In this technique from isolated protoplasts, cell, tissues, the organs are grown in *vitro*. These are grown on artificially prepared solid or liquid nutrient medium under aseptic and controlled conditions of light, humidity and temperature for achieving different objectives.

Plant tissue cultures are classified according to the type of in *vitro* growth i.e., callus and suspension cultures or the explants used for culture initiation, i.e., embryo culture, anther culture, etc. The plant material used for culturing is called explant. Totipotency is the inherent ability of plant cell to grow, divide, re-divide and give rise to a whole plant. German botanist Haberlandt (1902) developed the concept of *in vitro* culture.

It is essential that explants culture vessels, media, glass wares, working tables used for tissue culturing be made free from microorganisms. To maintain such aseptic condition sterilization is done.

3.3.3 Technique

The technique of tissue culture involves following steps.

1. Cleaning, sterilization of glass ware and instruments in an oven/ autoclave.
2. Selection and preparation of nutrient medium (Murashige and skoog medium MS medium) with known concentrations and proportion of different components.
3. Sterilization of the nutrient medium in an autoclave for 20 minutes under constant pressure i.e., 15 lb/inch^2 at a temperature of 105°C.
4. Preparation of plant material i.e., explants include isolation of explants followed by surface sterilization and rinsing with water.
5. Inoculation of the explant in the culture flask containing sterilized nutrient medium. Inoculation is done in the laminar air flow cabinet unit.
6. Incubation of the inoculated explants-in the flask cells of explants grow, proliferate to form callus, within 2-3 weeks.
7. Sub culturing of the callus (if the callus is to be maintained for longer period, callus is divided into 3-4 segments and transferred to fresh culture medium.)
8. Organogenesis-intitiation of rooting and shooting, that eventually leads to plantlet formation.
9. Hardening- Plantlets are transferred to polyethylene bags containing sterilized

soil and kept under low light and high humidity controlled conditions preferably in greenhouse for suitable period of time.

10. After hardening the plantlets from the polyethylene bag or pots are transferred to the field.

A - Parent plants
B - Explant surface sterilization
C - Explant dissected in pieces
D - Separated explant
E - Nutrient medium
F - Inoculation of rooting and shooting
I-Hardening, plantlets are transferred to pots or polybags kept in polyhouse
J - Plantlets from the pots or polybags are transferred to the field.

3.3.4 Various techniques in tissue culture :

Micropropagation :

This technique is used for the purposes of developing high quality clonal plants (a clone is a group of identical cells). This has the potential to provide rapid and large scale propagation of new genotypes.

Somatic cell genetics :

Used for haploid production and somatic hybridization.

Anther culture :

Plants produced through anther culture are haploids. Doubling the chromosomes without going into series of backcrossing can produce homozygous plants. This technique has profound application to plant breeder and shortens the time of breeding by half.

Embryo rescue :

Many important plants are difficult to propagate through seeds. They take a long time for seeds to germinate or the seeds do not germinate at all. This can be overcome through embryo culture. The seeds are surface sterilized and split open in aseptic condition and the tiny embryo is excised and planted in a nutrient medium and then grows to a complete plant.

Fig. 3.4 Activities in tissue culture technique
Courtesy : Kshitij biotech corporation, Karad, Dist. Satara

Organelle transfer :

In some cases, it may be desirable to transfer only organelles or the cytoplasm into a new genetic background. This may be achieved through the use of plant protoplasts. Chloroplasts have been transferred, and other organelles including nucleus may be transferred.

Transgenic plants :

Used for expression of mammalian genes or plant genes for various species. It has proved beneficial for the engineering of species that are resistant against viruses and insects.

3.3.5 Methodology involved in plant tissue culture :

This process involves the use of small pieces of a given plant tissue (plant of interest). Once the tissue is obtained, it is then cultured in the appropriate medium under sterile conditions so as to prevent various types of microorganisms from affecting the process.

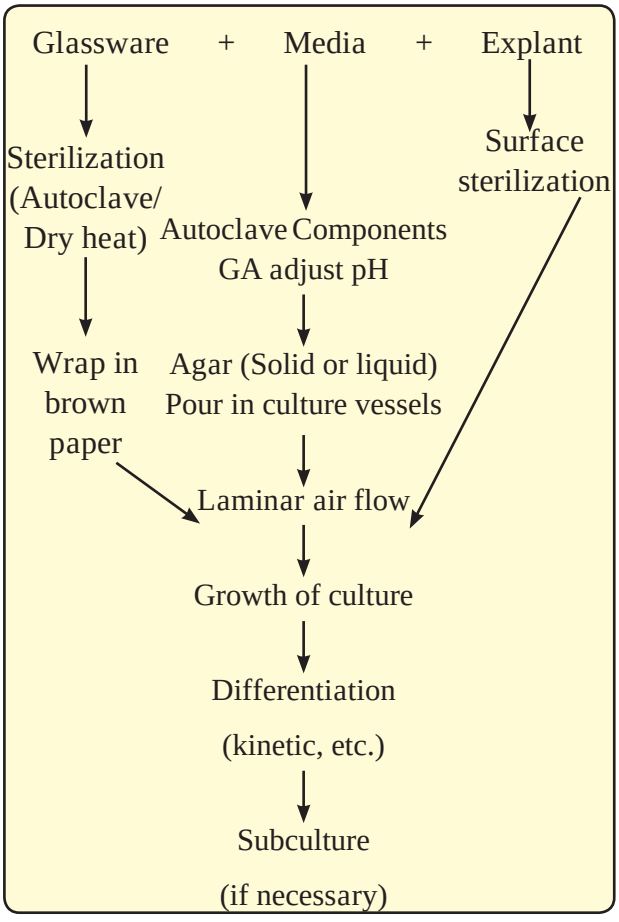


Fig. 3.5 Steps in tissue culture method

The following is a general procedure for plant tissue culture

A. Medium preparation

- The appropriate mixture (such as the MS mixture) is mixed with distilled water and stirred while adding appropriate amount of sugar and sugar mixture. Here, sodium hydroxide or hydrochloric acid is used to adjust the pH. Contents used here will depend on the plant to be cultured and the number of tissues to be cultured.
- Agar is added to the mixture, heat and stirred to dissolve
- After cooling, the warm medium is poured into polycarbonate tubes (to a depth of about 4 cm)
- With lids sitting on the tubes, the tubes are placed in a pressure cooker and sterilized for 20 minutes

B. Plant preparation

- Cut the plant part into small pieces (e.g. cauliflower can be cut to florets of about 1cm across). On the other hand, such parts as the African violet leaves can be used as a whole.
- Using detergent and water, wash the plant part for about 20 minutes.
- Transfer the plant part in to sterilizing Clorox solution, shake for a minute and leave it for 20 minutes.
- Using a lid, gently discard the Clorox and retain the plant part in the container and then cap the container

C. Transferring the plant material to a tissue culture medium

- About 70 percent alcohol should be used for sterilization of the equipment and containers used.
- Open the container and pour sterile water to cover half or the container
- Cover with a sterile lid again and shake the container for 2 to 3 minutes in order to wash the tissue and remove the bleach
- Pour water and repeat this, three times
- Using sterilized gloves, remove the plant part from the container and use a sterile petri dish

- Using a sterile blade cut the plant material to smaller pieces of about 2 to 3 mm across avoiding the parts that have been damaged by bleach
- Using sterile forceps, place a section of the plant in to the medium
- Depending on the plant used, it is important to check and find out how it should be placed in the medium
- Replace the lid/cap and close tightly

This procedure will result in the development of a callus, which then produces shoots after a few week. Once the shoots develop, then the plant section may be placed in the right environment (well lit, warmth, etc.) for further growth.

3.3.6 Advantages and Disadvantages

Advantages

1. Large scale multiplication and developing true plants in lesser time and small space.
2. Production of disease free plants round the year is possible irrelevant of climate.
3. Production of plants is possible.
4. Highly beneficial for plants where regular propagation is difficult e.g., crops like sugarcane, grapes, banana, etc.
5. In diocious fruit plants, production of female plants is possible through micro propagation e.g., papaya.
6. Production of homozygous plants by doubling haploids.
7. Helps in fast multiplication of rare species.
8. Embryo culture to overcome dormancy.
9. Raising plantlets such as orchids, which are difficult to multiply through seeds.
10. It produces disease free, drought resistant and salt tolerant plants.
11. The germplasm can be preserved for a long period of time.
12. This helps in obtaining uniform plant types than the original one.
13. Nitrogen fixation capacity can be introduced in the plants.
14. It helps in producing superior plant type than the original one.
15. The plants which are unable to grow under normal conditions can be grown easily with this technique.

Disadvantages

1. It is a difficult method for propagation and requires special technique and skill.
2. Expensive, sophisticated facilities, trained personnel and specialized techniques are essential.
3. High cost of production results from expensive facilities and high labour inputs e.g., shoot tip propagation requires much hand labour to transfer individual propagates.
4. High volume, more or less continuous distribution systems or adequate storage facilities to stock pile product is required.
5. Contamination or insect infestation can cause high losses in a short time.
6. Variable and off type individual can arise in the products emerging from micro propagation. Careful roguing, prior field testing of new products and continuous research and development are essential to decrease this risk.
7. Economics and marketing are key to the success of commercial production.
8. Decreases genetic variability.

Do you know ?

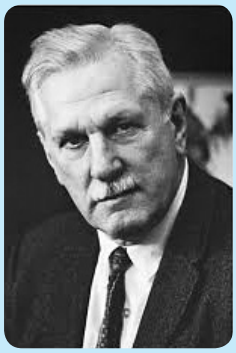
Plant materials should be sterilized so as to remove any bacteria or spores that may be present.

For plants, the medium culture acts as a greenhouse that provides the explant with the ideal environment for optimum growth. This includes being free of microorganisms, nutrients as well as the right balance of chemicals and hormones. Such media as BAP, TDZ are used while such hormones as IBA and IAA are used to induce growth.

Try this

- Visit the farmer having Bt cotton crop in his field and to know his experiences with such genetically modified crop.
- What are his experiences with non-Bt cotton.

Know the Scientist



Folke Karl Skoog (July 15, 1908 - February 15, 2001) was a Swedish born American Plant physiologist who was a pioneer in the field of plant growth regulators particully cytokinins Skoong was recipient of the National Medal of Science, 1991)

Toshion Murashige (1930) : Professor meritus of University of California. He is most widley known for his efforts in creating the plant tissue culture medium known as Murashigs and Skoog medium

Exercise

Q.1 Answer the following questions.

A. Select the appropriate alternative and complete the following statements.

1. Development of seed without sexual fusion refers to ---------
   a. Apomixis b. Parthenogenesis
   c. Apogamy d. Apospory
2. A flower containing both stamens and pistil is a ---------
   a. Staminate flower b. Pistilate flower
   c. Perfect flower d. None
3. Reproduction which does not involve fusion of male and female gametes is called ---------
   a. Apomixis b. Asexual
   c. Vegetative d. All above
4. Plant having male and female reproductive organs present in same flower is known as --------- flower.
   a. Dioecius b. Monoecious
   c. Unisexual d. Bisexual
5. The fusion of one of the two sperms with the egg cell, producing a diploid zygote is known as
   a. Reproduction b. Gametogenesis
   c. Fertilization d. Apospory.

B. Make the pair.

'A' Group	'B' Group
1. Staminate flower	a. Female flower
2. Papaya	b.Dioecious species
3. Pistilate flower	c. Bisexual flower
	d. Male flower
	e. Monoecious species

C. Find the odd one out.

1. Maize/Caster/Coconut/Papaya
2. Cutting/Layering/Grafting/ Parthenogenesis
3. Parthenogenesis/Apogamy/Apospory/ Budding
4. Budding/Grafting/Cutting/Seed
5. Fertilization/Sterilization/Medium/ Explant

D. Write True or False.

1. Asexual reproduction involves fusion of male and female gamets.
2. In Papaya, male and female flowers are present on two different plants.
3. By sexual reproduction combination of different genes from more parents in single hybrid plant is possible.
4. Bulbils are normal flowers.
5. Inoculation of the explant is necessary in tissue culture.

Q. 2 Answer in brief.

1. What is meant by plant tissue culture?
2. Define fertilization.
3. Name the types of apomixis.
4. Give the types of vegetative reproduction.
5. Define parthenogenesis.

Q.3 Answer the following questions.

1. What are the advantages of sexual reproduction.
2. What are the causes of parthenogenesis?
3. Describe in short apogamy.
4. Explain the techniques in tissue culture.
5. What is totipotency.

Q.4 Answer the following questions

1. What is the significance of sexual reproduction?
2. What is the significance of asexual reproduction?
3. Describe Apospory
4. What is Graffting?
5. What is meant by parthenogenesis?

Q. 5 Answer the following questions in detail.

1. Define sexual reproduction. What are its advantages and disadvantages?
2. Define asexual repreduction. Explain natural vegetative reproduction.

Q. 6 Answer the following questions in detail.

1. Write the stpes involved in technique of tissue culture.
2. Write the advantages and disadvantages of tissue culture.

Activity

- Visit a plant tissue culture lab and understand the various activities carried out.
- Dissect the flower and identify its various parts and draw its diagram.

Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola