Acclimatization and Establishment of Micropropagation Plants

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Abstract

Plant propagation by tissue culture is known as micropropagation. Four sequential stages are known in micropropagation system are as under:

- Establishment
- Multiplication
- Pretransplant
- Transplant

The basic procedure for stage one is to establish the explant in culture, screen for contaminated explant and allow excised plant unit to stabilized to the culture environment. Any plant tissue or organ could be used as explant source, but it success depend upon the culture system, species being cultured, and removal of the contaminant from the explants .The stage one provide large percentage of explants free from surface pathogens. In establishment stage a sterile explant is produced and planted on sterile basal medium, which include, semisolid medium, agar, mineral salts with minor and major element, sucrose use as a source of energy, vitamin supplements, hormone, plant growth regulator, cytokinin, auxin and gibberellin. Usually 4-6 weeks required to complete stage one.

Introduction

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Establishment

This stage is used to establish as sterile explant in culture. The various factors effect on this stage re:

a) Choice of explant

b) Elimination of contaminant from the explant

c) Culture condition like ingredients, light, temperature

d) Choice of explant export

e) Environmental conditions

The appropriate explant use can come from knowledge of the plant species and their cultivars.

a) Choice of explant

The size of explant varies from 1 to 5 mm meristem tip. In woody plant shoot-tip from dormant bud is utilize. Piece of leaves with veins present, bulb, scale, flowers, scapes and cotyledon, used to obtain explants.

Selection of explant and it success depend upon

- Hormone and media used
- Ingredients: agar, growth hormones, auxin, gibberellin
- Manipulations of shoot tip culture
- Special characteristic in culture
- Environmental factors

Establish the explant and induce multiple shoot development for further multiplication, based on:

- A-sexual formation of embroy's (embryogensis)
- The stimulation of axillary shoots
- The initiation of adentious shoots on excised shoots, leaves, bulb, scale, flower, scapes, cotyledon
- The initiation of callus from the cut surface

b) Elimination of contaminant from the explants

Pre-treatment

In this step explants are placed in petri dish on the surface of agar medium of basic salt and sugar without hormones. Phenolic and other substances should be exude because they inhibit development, charcoal, ascorbic acid, citric acid and added to the medium to prvent the effect. Use of antioxidants like ascorbic and citric acid is helpful.

The medium provides two functions. It provides basic nutritional ingredient to the explant and subsequent propagates. The hormones used for this purpose are auxin, cytokinin, gibberellins and proper sequence of hormones is important for particular plant growth like cytokinin stimulate shoot bud initiation on Douglas fire cotyledon but had to be remove of shoot. Like auxin stimulate root initiation on shoot, but may inhibit or reduce subsequent root growth. Embryogensis require auxin for introduction but embryo development occur in it absence .Establishment require Anderson medium in liquid form and inorganic salts ½ strength, sucrose 3%, 2iP 2mg/l and 1AA 0.5 mg/l.In 2-8 week axilary shoots developed and cut-off and transferred as they develop.

The Mineral composition of nutrient media (mg/liter)

KCl, $NaNO_3$, $MgSO_4 \times 7H_2O$, $NaH_2PO_4 \times H_2O$, $CaCl_2 \times 2H_2O$, KNO_3, $CaCl_2$, Na_2SO_4, $Ca(NO_3) \times 4H_2O$, NH_4NO_3, $FeSO_4 \times 7H_2O$, $MnSO_4 \times 4H_2O$, Kl, $NiCl_2 \times 6H_2O$, $CaCl_2 \times 6H_2O$, $Ti(SO_4)_3$, $ZnSO_4 \times 7H_2O$, $CuSO_4 \times 5H_2O$, $FeSO_4$, H_3BO_3, $FeCl_3 \times 6H_2O$, $Na_2MoO_4 \times 2H_2O$, $AlCl_3$, $Fe(SO_4)_3$, Feric Tertitate.

Organic constituents of media (mg/liter)

Sucrose, Glycine, Myo-inosital, 1AA, Cysteine, Vitamin B1(Thiamine), Nicotonic acid, Ethtylene diamine tetracetate (NaEDTA), Ca-D-panto themic , 2-4-D, Kinetin, Antoxidants are : ascorbic acid, citric acid, L-Cysteine, hydrochloride, 1,4-dithiothreitol, glutathione and mercaptoethanol.

A high cytokinin: auxin ratio promote shoot formation, while high auxin: cytokinin ratio promote root formation.2-4-D, A source of exogenous auxin in 1AA, NAA or IBA.Gibberelic acid is used for culture of shoot apices. Callus production need high concentration of auxin and low level of cytokinin. Adventious shoot initiation need high concentration of cytokinin and auxin.The medium varies according to cultivar and kind of explant used. For the development of adventitious shoots on explant need high level of cytokinin, while callus formation require high level of auxin.

The explant are divided in to small size then place in to nutrient medium for maximum growth. Explants can be derived from plant tissue which is in dormant phase. GA3 used to break dormancy. If top explant is larger size, growth will be rapid and better chances of survival. Immature flower buds are inflorescence are quite regenerative. A method with callus phase involvement is better due to high degree of genetically aberrant plants. Meristem-tip culture, heat treatment and various pathogen indexing test use to get virus free stock plants.

c) Control of pathogens through micropropagation

Are two types:

- External pathogens
- Internal pathogens

External pathogens

Reduction in surface contaminants begin with the control of stock plants use as a source of explants .Insect and mite population should be controlled. External pathogen are fungi, molds, bacteria, highest and other microorganisms. In order to control we must disinfect the explants. Humidity favor the growth of microorganisms, so the overhead watering sprinkling that increase humidity around the plants should be avoided. The plants growing out-of door covering shoots with plastic bag, need spray with fungicides. Disinfestation like calcium hypochloride and sodium hypochlorite are toxic to microorganisms and non toxic to plants. Alcohol (ethyl, methyl, or isopropyl) at 70-75 % used as disinfestant but highly toxic to plant material so it use is limited in rainless and sterilization. Antibiotic like streptomycin used to inhibit microorganism growth.

Internal pathogen are

- Virus and virus like organism
- Bacterial and Fungal contaminants

Virus and virus like organisms.

Some virus like dasheen mosaic virus in dieffenbachia seriously inhibited the growth in culture. The extreme meristem-tip of shoot is utilize to separate the explants from any virus in the rest of plant. Some time use heat treatment for this purpose.

Bacterial and fungal contaminants

Bacterial make contaminant like, bacillus subtilis, erwinia or pseudomonas, these contaminants inhibit growth and rooting potential .Culture indexing also used to eliminate those explants showing positive results for the presence of pathogens.

d) Elimination of contaminants

Sterilization is important for successful micropropagtion .The sterilizing agent , should easily removable from the surface of the explant .The standard techniques for sterilization of culture media and culture equipment are outclaving at 1.05 kgcm2(15 ib/in2(121C°) for 15 minutes.

Thermo-labile substances (Vitamins and growth regulators) are sterilized by filtering a solution of substances through a bacterial proof sintered glass filter having a pore size of 0.22mm.

Explants of some species contain endogenous substances that exclude from the cut surface in to medium and inhibit development, antioxidants such as ascorbic acid or citric acid used for washing solution in the medium. Disinfecting and sterilization done by washing and using fungicide solution. After washing a 10-50% commercial color or laundry bleach 0.5-5.25 % sodium hypochlorite used for sterilants.
During shoot-tip propagation is cut the shoot in the short pieces several centimeter long and dip them in to 70-90% alcohol, for sterilization, antibiotics also used to control bacterial growth. Detergent may be polyoxythylene sorbitan manolaurate used to sterilant and help in breaking the surface tension between water and plant tissue.

Chemical use for surface steriliant for treating explants prior to aseptic culture use:

- Calcium chloride
- Sodium hypochlorite
- Bromine water
- Hydrogen peroxide
- Mercuric chloride
- Absolute alcohol
- Silver nitrate
- Antibiotics.

Disinfectant may be ethanol, calcium hypochloride, hydrogen peroxide, silver nitrate, bromine water and mercuric chloride. Activated charcoal used as absorbent material used in the medium. Internal contaminates are removed by the use 10mg/liter of benomyl or benlate before disinfestation with commercial bleach. Cefotaxine (Sigma chm) antibiotic helps in shoot proliferation and destroy the growth of bacteria. During this process tissue get brown, following ways can prevent this browning of tissue:

- Removal of phenolic compound produced.
- Modify Redox potential
- Inactivating Phenolase enzymes
- Reduce Phenolase activity/substrate availability
- Reducing antioxidants.

e) The major environmental factors are as under …

Light, temperature and relative humidity is 100%

Light

The light is needed to regulate morphogenetic processes, such as initiation of roots, formation of shoot, chlorophyll and asexual embryogenesis but low light intensity (300-1000 lux) is required to maintains the growth of callus tissue an early organogenesis, while high intensity (3,000-10,000 Lux) light is required for rapid growth of plantlets. Duration of light period varies with different crops.

Light quality effect on organogenesis like blue light fervor shoot initiation, root initiation is stimulated by red lights but balance exposure to red and blue light is essential for balance root and shoot formation. For this purpose luminescent lamp and incandescent lamps used for this purpose.
Light and temperature are not usually critical in establishment stage usually 20-25 C° temperature and light intensity about 1000 Lux is used. Growing stock plants need controlled light and temperature with proper physiological stage give good flush of growth .

Temperature

Best temperature is 25C° +2.3C° for organogenesis. Alternating temperature during day and night is needed for organ formation. Higher temperature in day is essential for cambium while lower night temperature for differentiate the cambium in to root primodium. Changes in temperature, day light , light intensity, water availability through out the year effect on the level of carbohydrate, protein and growth substances in the stock plants thus subsequently affecting the response of the explant in vitro.

f) Handling the stock plants

Stock plants handling depend upon, to reduce the potential for contaminants by fungi, bacterial, virus and other pathogens, the other is the physiological condition of the explant, some artificial light help in new flushes of growth. Proper handling of stock plants may be important to get good regeneration and reduce contamination

Factors affecting success in micropropagation

Genotype

Even with a species, some cultivar response better than others and are apt to be selected for microprogation, with the time improve genotype and improve procedure also developed. In general those plants are easier to propagate conventionally are easiest to microproate. Woody perennials due to their juvenility status is difficult but confer species due to axillary bud characteristic are easy to propagate. The explant grow by elongation of the main terminal shoot with limited axilary shoot to produce a mass of microshoots depending upon the apical dominance of particular kind of plant.

Culture mass is divided after 2-4 weeks and subculture on fresh medium. Division and subculture is reated after shot interval .Stabilization needed subculturing to produce uniform, well-growing culture.

Example: Apple (Malus Pumila)

Various procedures are used for its micropropagation. In one case shoot tip growing in simplified medium one week before transferring, other method grow shoot-tip at length of 10-15 mm in 15ml liquid media, 125 Erlenmeyer for 2-4 days then transfer them to agar medium, one which they are planted horizontally to half their thickness. Polyvincy-prolidine used as agar medium , 4-8 weeks. The medium include BA(1mg/l), IBA, 0.1mg/l and GA 0.1-0.5 mg/l.

Inducing adventitious shoots, on root cutting also useful. The young tissue like terminal or axillary shoot-tip or tip of adventitious shoots, regenerate better than mature tissue. Turmeric and ginger used micropropagation show high multiplication rate and high percentage of field establishment.

Juvenility (epigenetic) effects

The micropropagation sequence of subculturing appears to rejuvenating process which ultimately leads to increased competence for rooting. In woody plant, rejuvenation to induce juvenile growth.

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