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Meristem Culture for Crop Improvement: An overview

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ABSTRACT: Viral infections are systemic, being pervasive in the entire affected plant. Heat therapy is a procedure that is used for ridding infected plants of viral infections. After heat treatment, subsequent new growth may be free of viruses. More precisely, meristems dissected from leaf and shoot primordia are more often free of viruses even when the plant is infected. Tissue culture technology is used to nurture the excised meristematic tissue into full plants that are free from viruses. The virus-free plants are used to produce more materials (by micropropagation) for planting a virus free crop. It should be pointed out that virus elimination from plants does not make them virus resistant. The producer should adopt appropriate measures to protect the crop from infection. Hence, this review

KEY WORDS: Viral infections, Tissue Culture, Meristem Culture, shoot tip

I. INTRODUCTION:

A group of identical cells which are in a continuous state of cell division. Some of the cells from the meristematic tissue stops dividing and exhibit certain changes to become permanent tissues of the plant. This change from meristematic to permanent state is called as differentiation. The rest of the cells in the meristematic tissues persists their meristematic activity. The meristematic tissues are self-perpetuating.

II. FEATURES OF MERISTEMATIC CELLS

The meristematic cells may be round, oval, polygonal or rectangular in shape. These cells are arranged closely without intercellular spaces. They have dense cytoplasm with large nucleus. They have smaller vacuoles scattered throughout the cytoplasm. Their cell walls were thin, elastic and made up of cellulose.

III. CLASSIFICATION OF MERISTEMS

Based on its position, meristematic tissues are classified into three types:

I. Apical meristem

II. Intercalary meristem

III. Lateral meristem

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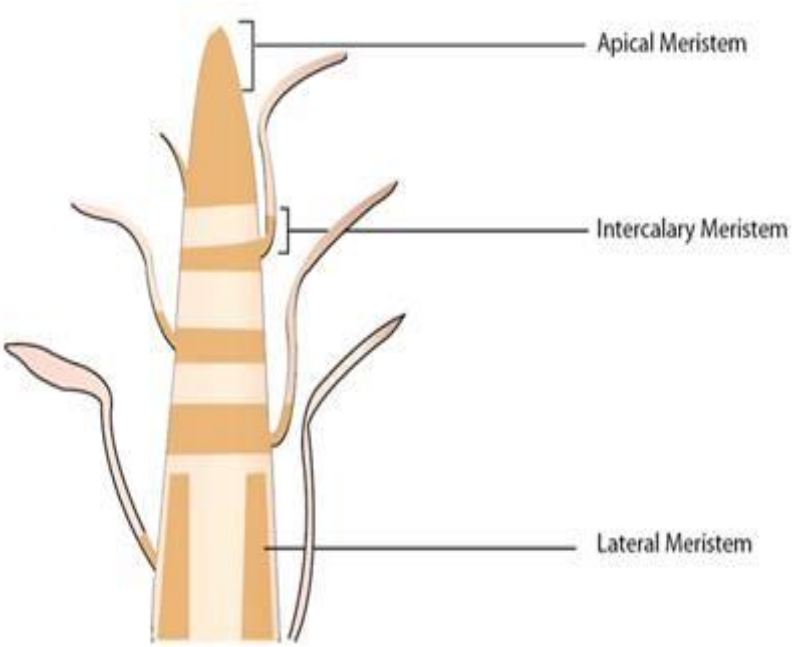

Figure: Longitudinal section of shoot- showing the positions of meristems

A.APICAL MERISTEM: Apical meristem is found at the tips of the roots, stems and branches. It plays a major role in the increase of plant length. It is divided into three zones namely protoderm, procambium and ground meristem. Protoderm gives rise to epidermal tissue. Procambium gives rise to primary vascular tissue and ground stem gives rise to cortex and pith. Apical meristem with two to three leaf primordia constitutes the apex.

B. INTERCALARY MERISTEM: Intercalary meristems is present in the nodal region and is very prominently found in the monocotyledons, e.g., grasses. The name itself represents that intercalary meristems are found in between the permanent tissues. It is responsible for the elongation of nodes.

C. LATERAL MERISTEM: The meristem that is present along the longitudinal axis of stem and root is called lateral meristem, for example, vascular cambium and cork cambium. It produces secondary meristem tissues, which result in the thickening of stem and root.

IV. ESTABLISHMENT OF PATHOGEN FREE PLANTS

The plants infected with bacteria and fungi can be treated by bactericidal and fungicidal compounds, there is no commercially available treatment to cure virus-infected plants. A large number of viruses are not transmitted through seeds. Therefore, it would be possible to obtain virus free plants from infected individuals by using seeds as propagules. However, genetic variation often occurs from the sexually reproduced plants when propagated by seeds. Generally, clonal multiplication of cultivars can be achieved by vegetative propagation. However, where the entire population of the clone is infected the only way to obtain pathogen-free stock is to eradicate the pathogen from vegetative parts of the plants and regenerate full plants from such tissues. Once pathogen free plants are obtained, they can be multiplied indefinitely under conditions which would protect them from chance reinfection.

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V. *IN VITRO* MERISTEM AND SHOOT-TIP CULTURE

A. EXPLANT TERMINOLOGY: The scientist who have attempted to recover pathogen-free plants through tissue culture techniques have indiscriminately designated the explants required to initiate cultures as 'shoot-tip', 'tip-meristem' and 'meristem-tip'. The apical meristem of a shoot is the portion lying distal to the youngest leaf primordium, it measures up to about 100µm in diameter and 250µm in length. The apical meristem together with one to three young leaf primordia, measuring 100-500µm, constitutes the shoot-apex (Figure 7.2A and B). Although the chances of eradicating viruses are higher through 'meristem' culture, in most successful report's virus-free plants have been raised by culturing 100-1000µm long explants which could be according to the above definition is referred as 'shoot-tip'. To distinguish it from the *in vivo* technique of propagation through shoot-tip cuttings, the term 'meristem-tip' culture has been preferred for *in vitro* culture of small hoot-tips.

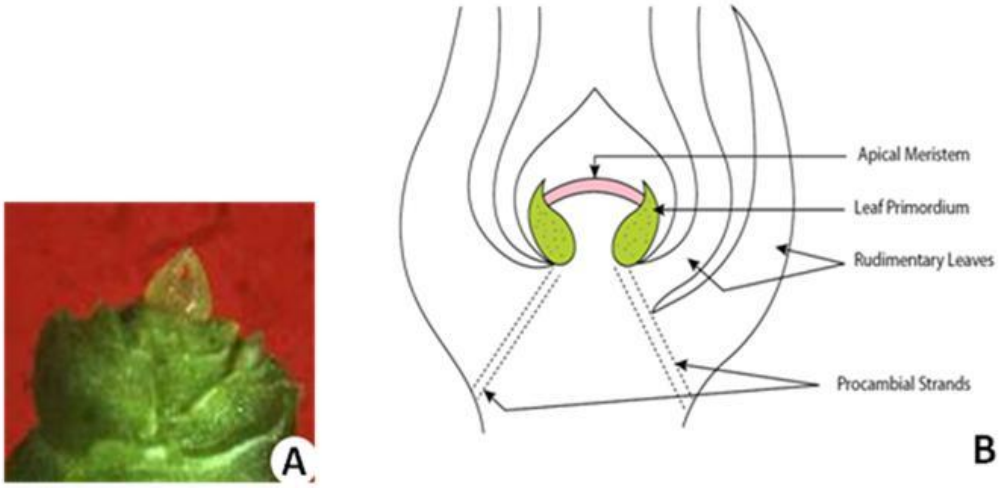

Figure: A shoot-tip explant; **B.** A cross section of shoot-tip meristem.

B. REASONS FOR ESCAPE OF MERISTEM FROM VIRUS:

It is well known that the distribution of viruses in plants is uneven. In infected plants the apical meristems are generally either free or carry a very low concentration of the viruses. In older tissues the virus titer increases with increasing distance from the meristem-tips. The reasons proposed for the escape of meristem from virus invasion are:

(a) Viruses readily move in a plant body through the vascular system which is absent in the meristem.

(b) The alternative method of cell-to-cell movement of the virus through plasmodesmata is rather too slow to keep pace with the actively growing tip.

(c) High metabolic activity in the actively dividing meristem cells does not allow virus replication.

(d) The 'virus inactivating systems' in the plant body, if any, has higher activity in the meristem than in any other region. Thus, the meristem is protected from infection.

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(e) A high endogenous auxin level in shoot apices may inhibit virus multiplication.

VI. FACTORS AFFECTING ERADICATION OF VIRUS THROUGH MERISTEM TIP CULTURE

Culture medium, explant size and incubation conditions affecting plant regeneration from meristem-tip cultures have pronounced effect on virus eradication. Besides, thermotherapy or chemotherapy and physiological stage of the explants also affect virus elimination by shoot-tip culture.

A. CULTURE MEDIUM: The nutrients, growth regulators and nature of the medium highly influence the development of virus free plants from meristem tip cultures. Maximum success is achieved from Murashige & Skoog's (MS) medium which promoted healthy, green shoot development compare to other nutrient media. The main reason for the suitability of medium for meristem-tip culture could be the presence of high levels of K^+ and $NH4^+$ ions. There is no critical assessment on the role of various vitamins or amino acids but sucrose or glucose is the most commonly used carbon source in the medium, at the range of 2-4%, to raise virus free plants from meristem-tip cultures.

Large meristem-tip explants, measuring 500µm or more in length, may give rise to plants even in the basal medium but generally the presence of an auxin or a cytokinin or both plays a major role in the development of excised apical meristem. In angiosperms, the meristematic dome in the shoot-tip does not synthesize auxin on its own, but it is supplied by the second pair of youngest leaf primordia. Therefore, for development of excised meristem in culture, without the leaf primordia, requires the supply of exogenous auxin. The plants requiring only auxin must have a high endogenous cytokinin level in their meristems. Among auxins, the use of 2,4-D should be avoided which promotes only callusing. NAA and IAA are widely used auxins and NAA being preferred due to better stability. The role of GA3 is also emphasized by few authors which is suggested to promote better growth and differentiation and suppresses callusing from meristem explants. Both liquid and semi-solid media have been tried for meristem–tip culture but, agar medium is generally preferred.

B. EXPLANT SIZE: The survival of the meristem tips, under the controlled condition, is determined by the size of the explant. The larger the explant, the greater are the chances of plant regeneration. However, the survival of the explants cannot be treated independent of the efficiency with which virus elimination is achieved that is inversely related to the size of the explant. Thus, explants should be small enough to eradicate viruses and large enough to be able to develop into a complete plant. Besides the size of the explant, the presence of leaf primordia influences the ability of the meristems to form plants. In some plants it is essential to excise shoot meristems with two to three leaf primordia. Smith and Murashige (1970) have suggested that leaf primordia supply auxin and cytokinin to the meristem necessary for its growth and differentiation. In a culture medium containing essential growth regulators, the excised meristems domes develop bipolar axes very quickly during reorganization (Figure 7.3). Once the root-shoot axis is established further development follows the same pattern as that of seedlings.

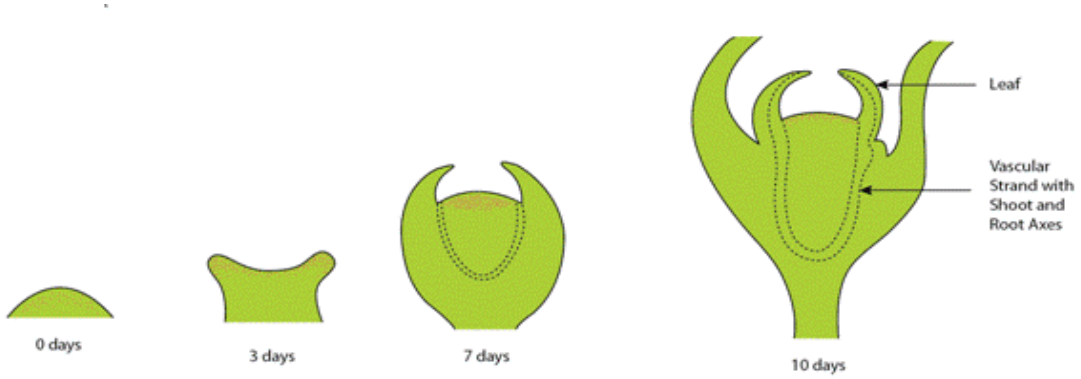

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Figure: Schematic representation of development of bipolar axes by meristem culture

C. STORAGE CONDITIONS Generally, light incubation of meristem tip culture is found better than dark incubation. The light intensity could range from 100 lx to 4000 lx which should increase in succession as the differentiation of meristem explant progresses. There are no clear information on the effect of temperature on regeneration of plants from excised meristem tips. The cultures are normally stored under room temperature (25±2°C) conditions.

D. PHYSIOLOGICAL CONDITIONS OF THE EXPLANT Meristem-tips should be collected from actively growing buds. In few cases, the tips taken from terminal buds proved better than those taken from axillary buds. Seeing the higher number of axillary buds present per shoot, in majority of the reports axillary buds were utilized as explants to increase the overall production of virus-free plants. The time excision of buds is also critical, especially for the trees with periodic growth. For example, in temperate trees the growth of the plant is limited to only a very short period in the spring and afterwards dormancy starts. In such cases, the meristem-tip cultures can be raised during spring only for increased success rate.

E. THERMOTHERAPY Often, apical meristems are not always free of virus and it can't be considered as a universal occurrence. There are certain viruses like, Tobacco Mosaic Virus (TMV), Potato Virus X (PVX) and Cucumber Mosaic Virus (CMV), which invade the meristematic region of the growing tips and interrupts the growth of the meristematic tissue. In such cases also it has been possible to obtain virus-free plants by combining meristem-tip culture with thermotherapy. In this technique, first the mother plants are exposed to heat treatment before excising the meristem-tips or, alternatively, shoot-tip cultures are exposed to high temperature regimes (35°C-40°C) for certain duration (6h to 6 weeks) to obtain virus free plants. In the later case, continuous exposure to very high temperature causes deterioration of the host tissues. The first procedure of treating the mother plant has added advantage where larger explants can be taken from the treated stock and thus, favors relatively higher chances of the tip survival.

F. CHEMOTHERAPY Chemotherapy is the treatment of an ailment by chemicals especially by killing micro-organisms. It will not eradicate the virus completely. However, a large number of antibiotics, growth regulators, amino acids, purines and pyrimidines can be tested for inactivation of viruses. A nucleotide analogue ribavirin has been found to be the most efficient viracide for plant viruses. This broad spectrum antiviral agent, effective against both plant and animal, was reported to eliminate PVY, CMV and TMV from tobacco explant cultures, Chlorotic Leaf Spot Virus (CLSV) in apple cultures when incorporated into the medium. Vidarabine (adenine arabinoside) and antiserum are also known to reduce the titre of viruses. The effectivity of the compound may vary with the virus and the host genotype.

G. VIRUS ELIMINATION THROUGH CALLUS CULTURE It is a general observation that not all the cells in a calli uniformly carry the pathogen when raised from infected tissues. The two possible reasons for the escape of some cells of a systematically infected callus from virus infection are: (a) virus replication is unable to keep pace with cell proliferation, and (b) some cells acquire resistance to virus infection through mutagenesis. Therefore, it is possible to raise virus-free plants from infected shoot-tip calli. However, genetic instability of cultured cells and lack of plant regeneration in callus cultures of some plants poses the limitations of using calli for virus elimination.

H. VIRUS INDEXING Even after subjecting the meristem-tips to various treatments favoring virus eradication, only a proportion of the cultures yield virus free plants. Therefore, it is required to test all plants, regenerated through meristem-tip or callus cultures, for specific viruses before being used as mother plant to produce virus-free stock. The individual plants consistently showing negative results for virus titre can be marked as ‘virus tested' for specific virus/es and can be released for commercial purposes. The following tests can be performed for virus testing:

i. The simplest test for the presence or absence of viruses in plant tissues is to examine the leaves and stem for the visible symptoms characteristic of the virus.

ii. Another test is the sap transmission test or ‘bioassay test' or ‘infectivity test'. It is a very sensitive test and can be performed at a commercial scale. To perform this, ground the test leaves in equal volume (w/v) of 0.5M phosphate

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buffer using a mortar and pestle. Leaves of the indicator plant (a plant very susceptible to specific viruses), dusted with 600-grade carborundum, are swabbed with the leaf sap from the test plant. After 5 min the incubated leaves are gently washed with water to remove the residual inoculum. The inoculated indicator plants are maintained in a glasshouse, separate from other plants. It may take several days to several weeks, depending on the nature of virus and the virus titre, for the symptoms to appear on the indicator plants. It is used to detect some viruses and viroids but is a slow process requiring several days to months.

iii. The third method, enzyme-linked immunosorbant assay (ELISA), is more rapid serological test which allows quick detection of important viruses. It relies on the use of antibodies prepared against the viral coat protein, requires only a small amount of antiserum and can be performed with simple equipment. However, it is not applicable to viroids and viruses which have lost their coat proteins

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