Callus culture

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Plant callus (plural *calluses* or *calli*):- is a mass of unorganized <u>parenchyma</u> cells derived from plant tissue (explants) for use in biological research and biotechnology. In plant biology, callus cells are those cells that cover a plant wound.

Callus cells are not necessarily genetically homogeneous because a callus is often made from structural tissue , not individual cells

Callus formation is induced from plant tissues after surface sterilization and plating onto *in vitro* tissue culture medium. Plant growth regulators, such as <u>auxins</u>, <u>cytokinins</u>, and <u>gibberellins</u>, are supplemented into the medium to initiate callus formation or <u>somatic</u> <u>embryogenesis</u>.

Callus:

- **Definition:** It is an unspecialized and unorganized, growing and dividing mass of cells,.
- During callus formation there is some degree of dedifferentiation both in morphology and metabolism, resulting in the lose the ability to photosynthesis.
- Compact callus
- Friable callus





Plant callus is usually derived from <u>somatic</u> tissues. The tissues used to initiate callus formation depends on plant species and which tissues are available for <u>explant</u> <u>culture</u>.

The cells that give rise to callus and somatic embryos usually undergo rapid division or are partially undifferentiated such as <u>meristematic</u> tissue.

Plant hormones are used to initiate callus growth.

Morphology

Specific <u>auxin</u> to <u>cytokinin</u> ratios in plant tissue culture medium give rise to an unorganized growing and dividing mass of callus cells.

Callus cultures are often broadly classified as being either compact or friable. Friable calluses fall apart easily, and can be used to generate cell suspension cultures. Callus can directly undergo direct <u>organogenesis</u> and/or <u>embryogenesis</u> where the cells will form an entirely new plant.

- The explant is commonly cultured on a nutrient medium solidified in agar. Explants from most species of plants may be induced to divide in an unorganized manner on specifically formulated nutrient media
- An undifferentiated mass of cells, known as callus (plural, calli), is formed within 4 to 8 weeks.
- The callus may be divided, with clusters of cells transferred to fresh agar media to form subcultures. Repeated subculturing of the callus permits rapid multiplication of the cultured material.

- Plant regenerability may decline, and genetic stability of the plant material may be altered, with successive subculturing.
- Callus cultures are incubated under aseptic conditions, normally in dim light, with temperatures around 25°C.

Callus induction

A callus cell culture is usually sustained on gel medium. Callus induction medium consists of agar and a mixture of <u>macronutrients</u> and <u>micronutrients</u> for the given cell type.

Murashige and Skoog medium, White's medium (woody plant medium).

Vitamins are also provided to enhance growth such as B5 vitamins . For plant cells, enrichment with <u>nitrogen</u>, <u>phosphorus</u>, and <u>potassium</u> is especially important.

Stages of initiation callus

Callus

- Induction stage

Explant

- Cell division stage
- Differentiation stage







Callus cells deaths

Callus can brown and die during culture, but the causes for callus browning are not well understood.

Browning has also been associated with oxidation and phenolic compounds in both explant tissues and explant secretions.

Uses

Nevertheless, callus cells are often considered similar enough for standard scientific analysis to be performed as if on a single subject. For example, an experiment may have half a callus undergo a treatment as the experimental group, while the other half undergoes a similar but non-active treatment as the control group

Plant calli can differentiate into a whole plant, a process called regeneration, through addition of plant hormones in culture medium. This ability is known as totipotency. Regeneration of a whole plant from a single cell allows researchers to recover whole plants that have a copy of the transgene in every cell. Regeneration of a whole plant that has some genetically transformed cells and some untransformed cells is called a chimera. In general, chimeras are not useful for genetic research or agricultural applications.

- Genes can be inserted into callus cells using biolistic bombardment, also known as a gene gun, or Agrobacterium tumefaciens. Cells that receive the gene of interest can then be recovered into whole plants using a combination of plant hormones. The whole plants that are recovered can be used to experimentally determine gene function(s), or to enhance crop plant traits for modern agriculture.
- Callus is of particular use in <u>micropropagation</u> where it can be used to grow genetically identical copies of plants with desirable characteristics

Application of Callus Culture

1. The whole plant can be regenerated in large number from callus tissue through manipulation of the nutrient and hormonal constituents in the culture medium which is called as organogenesis or morphogenesis. Similarly, callus can be induce to form somatic embryo which can gives rise to whole plant.

2 . Callus tissue is good source of genetic or karyotypic variability, so it may be possible to regenerate a plant from genetically variable cells of the callus tissue. 3 . Cell suspension culture in moving liquid medium can be initiated from callus culture.
4 . Callus culture is very useful to obtain

4 . Callus culture is very useful to obtain commercially important secondary metabolites. If a bit tissue from a medicinally important plant is grown in vitro and produced callus culture, then secondary metabolites or drugs can be directly extracted from the callus tissues without sacrfting the whole plant.

5. Several biochemical assays can be performed from callus culture.