The Cannabis Tissue Culture Glossary

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Agar

Agar is the most common gelling agent used in plant tissue culture. It is made from red algae and added as powder to tissue culture media. It dissolves during autoclaving and the media will solidify into a gel after cooling. In Cannabis tissue culture agar is added at 0.75-1.5% (w/v), depending on the desired firmness of the gel. The concentration of agar in the media also influences humidity in the culture vessel.

Apical Dominance

Apical dominance refers to the suppression of axillary buds by the tip of a shoot or branch. Cannabis seedlings or clones will grow one main stem with few branches unless topped. This allows the young plant to quickly gain height and grow closer to the light. Once the tip is removed, the buds in the leaf axils grow into branches.

Apical dominance is caused by auxin plant growth regulators that are produced in the apical bud and are transported downwards in the phloem. When the apical bud is removed, the suppression ends, and axillary buds grow into branches.

Artificial Seeds

Also called synthetic seeds or clonal seeds, artificial seeds contain sterile explants in alginate-based nutrient gel that is encapsulated by a calcium shell. They are prepared by first immersing the explants in sodium alginate solution containing nutrients, an energy source, and plant growth regulators. The alginate solution has the same function as endosperm in a seed. Droplets of the alginate solution containing one explant each are then dropped into a calcium chloride solution. The alginate and calcium will form a semi-rigid shell. The artificial seeds can be stored in liquid in a refrigerator. They "germinate" when placed on agar media under tissue culture conditions. The conditions for preparing and storing artificial seeds still need to be optimized. In our laboratory the seeds remained viable for up to 90 days. However, the recovery rates declined rapidly with storage time. Methods for storing artificial Cannabis seeds in liquid nitrogen are under development.

The main difference between artificial and natural seeds is that the embryo in a natural seed is derived from a zygote that contains one set of chromosomes from each parent. Artificial seeds contain explants that are clones of the mother plant. The explants can be meristems, buds, nodes, or somatic embryos. Applications for artificial seeds are storage, shipping of genetic assets, and mass-propagation.

Autoclave

An autoclave is a type of pressure cooker for sterilizing liquids, tools, biological waste, and culture vessels. In autoclaves water vapor is pressurized to 15 psi (103 KPa) and heated to 250oF

(121 oC). Most autoclave cycles are 20-30 minutes long. Autoclaving neutralizes fungal and bacterial spores that can survive boiling, and dissolves agar used in solidified culture media.

Auxin

Auxins are distributed throughout the plants and transported through the vascular system. Auxins travel from shoot tips downward towards the roots. Clones can root without addition of rooting agents when auxins accumulate at the base of the cuttings. Cannabis clones and seedlings first grow upwards with little branching because auxins produced in the shoot tips and transported downward through the phloem suppress buds in the lower nodes, a process called apical dominance. Topping removes the shoot tip and branches develop.

Auxins work as antagonists or in coordination with other PGRs and the ratios determine whether shoots, roots, or callus develop in tissue culture.

The most common plant-based auxin is indole-3-acetic acid (IAA), the most common synthetic one is 2,4-dichlorophenoxyacetic acid (2,4 D), which is also used as herbicide to selectively kill broad leaved weeds in lawns.

Bud

An immature, dormant shoot. Buds are formed in leaf axils or on shoot or branch tips (apical or terminal buds). They contain meristem and immature leaf and stem tissue. Buds grow into shoots or branches when not suppressed by apical dominance (clones or seedlings before topping) or environmental conditions (trees and bushes in winter).

In Cannabis cultivation the term is used to describe the clusters of female flowers that contain most of the trichomes. The botanical term for these clusters is inflorescence.

Callus

Callus is a cluster of non-differentiated cells. In tissue culture calluses are irregular clumps that grow from explants and keep growing as long as the nutrients and carbon source in the media are not depleted. Callus can be green, brown, or white. Callus cultures can be started from almost any type of explant and stimulated by combinations of cytokinines and auxins. Calluses can be compact or friable. In friable calluses the cells are more loosely connected than in compact calluses. Friable calluses break up easily and can appear to be made of small bubbles. Callus cells can develop into somatic embryos when stimulated by combinations of plant growth regulators and environmental conditions.

Chimera

Chimeras are plants (or animals) with genetic changes in parts of their bodies. These changes are the result of random mutations that occur in dividing cells, such as meristem cells. The cell that carries the initial mutation will pass it on to all its progeny cells. How much of the plant is affected depends on when and where the mutation occurs. Mutations in apical or axillary meristems can be present in whole branches and shoots. Chimeras frequently occur when treating growing tissue with colchicine or oryzalin to induce polyploidy. The resulting plants can be a mosaic of diploid, tetraploid, and octoploid tissues. Common examples of chimeric Cannabis are

leaf lobes with green and yellow halves left and right of the midrib.

Cloning

Cloning is the process of generating genetically identical progeny of a plant or animal, called clones. In contrast to sexual propagation through seeds, it does not involve genetic recombination through formation of germ cells (pollen/sperm and egg cells) and fertilization. In plants clones are produced through cuttings or tissue culture.

Genetic variation in clones only occurs through random mutations, such as deletion, addition, or replacement of single or multiple nucleotides in the DNA sequence. These happen through mistakes in the DNA replication process when cells divide or environmental factors such as radiation. Mutations are affecting only the cells that originate from the cell that originally mutated. When mutations occur in apical or axillary meristems entire branches or stems can be affected.

Cloning produces predictable and consistent genetic copies of the mother plant. Changes in yield or potency are due to environmental factors and reversible. One advantage in Cannabis cultivation is that all clones from a mother plant are female.

Diseases and pests affecting the mother plants are passed on to the clones. This carryover can be limited in conventional cloning by dipping the cuttings in pesticides but endophytes (fungi and bacteria living inside plant tissue) viruses, and burrowing mites will still be present in the clones. Tissue culture can eliminate most carryovers of pathogens.

Propagation through seeds also excludes many pathogens and pests but produces a genetically variable offspring.

CRISPR-Cas9

The CRISPR-Cas9 gene editing system is a molecular biology method that allows for inserting or deleting DNA at specific locations of a target cell. It is based on a bacterial antiviral mechanism that creates a DNA memory (CRISPR) of past virus infections and defends against repeat infections by cleaving the virus' DNA (Cas9).

The first step in the process is to construct a DNA fragment that encodes the Cas9 nuclease (the protein that cuts the DNA), an RNA sequence that recognizes the target location, and the DNA sequence to be inserted into the host genome. This construct is then introduced into the target cells using a carrier, usually a circular DNA that multiplies in bacteria. This process is called transfection. The host cell will then read the information and produce a Cas9/RNA complex that will find the target location in the host's genome and cut the DNA. The host cell's DNA repair mechanism will then either reconnect the break or introduce the DNA sequence encoded in the construct. The result is a cell that contains one or more artificially created gene(s) or a repair site that inactivates an existing gene.

Cryopreservation

Cryopreservation refers to different methods of maintaining cultivars, or strains for extended time. There are several methods that have been established for different crops that are being used or evaluated for Cannabis.

Slow growth culture. Keeping cultured tissue refrigerated and under low light slows down shoot development and extends the expiration of the culture media. Less subcultures are needed for long-term maintenance.
Artificial seeds. Explants can be stored as artificial seeds in liquid or under moist conditions at low temperatures.
Storage in liquid Nitrogen. Maintaining cells or tissue at -321oF (-196oC) can preserve viability for years. This has been shown for some crops and is routinely applied for animal cells and tissue. The process requires that water in the frozen cells does not form ice crystals. The explants to be frozen must be treated to replace enough of the cellular water with glycerol or infuse the cells with a non-lethal antifreeze. These procedures are still under development for Cannabis.

Culture Media

Liquid or solidified blend of nutrients and plant growth regulators (plant hormones), carbon source (usually sucrose), and gelling agent (for solid media). Culture media are prepared by blending the ingredients in water, adjusting the pH, and autoclaving. Additives that are unstable in the autoclave must be added to the media after autoclaving. Solid media is poured into sterilized culture vessels and gels while cooling.
The concentrations and ratios of plant growth regulators determine whether the culture will produce callus, shoots, or roots. The carbon source is needed to “feed” the culture until shoots and leaves have developed and photosynthesis begins. Other macro- and micro-nutrients provide the necessary components for growth. Most Cannabis nutrients are

modifications of standard blends that were developed for other plant species.

Cytokinin

Cytokinines are a group of chemicals that influence cell division and shoot formation. Various natural and synthetic cytokinins are used in tissue culture to stimulate shoot growth and branching. They also affect the short- and long-distance transport of auxins and have shown to have anti-microbial properties that increase resistance. Cytokinins are mostly produced in growing tissue and roots.
Both the types and the concentration of cytokinins determine the effects in tissue culture. Combinations of different cytokinines are usually used. The most common ones in Cannabis culture are zeatin, kinetin, 6-benzylaminopurine (BA), and thidiazuron (TDZ).

Epigenetic Change

Epigenetic changes are DNA modifications that do not involve changes in the nucleotide sequence but affect how genetic information is converted into biochemical or structural traits. When cells divide and develop into specific cell types, different genes are activated or silenced. This regulation involves proteins that bind to specific DNA sequences or changes to the DNA itself, like methylation. Such modifications can be part of normal plant development or occur in response to environmental stress or pathogen infection. Most epigenetic changes are stable and passed on through seeds and clones. Epigenetic changes accumulate over time and can have negative effects on plant vigor or productivity. They are one of the

causes of gradual change of Cannabis strains over successive cloning cycles. Some epigenetic changes can be reversed through tissue culture, which is one of the reasons that tissue culture propagation can often restore strains that have declined over time.

Laminar Flow Cabinet

A laminar flow cabinet, or hood, provides a near sterile work environment by maintaining an evenly distributed flow of filtered air. The HEPA (high efficiency particulate air) filter is usually located on top of the cabinet. Filtered air is blown into the partially enclosed workspace below, preventing airborne contamination. In contrast to a fume hood air flows toward the user and does not protect from contamination in the material being worked on, such as moldy tissue or media.
Sterilization and washing of tissue culture explants, handling autoclaved culture media, opening of culture vessels, and transplanting of cultured tissue are done in the laminar flow cabinet.

Explant

Explants are cuttings that are used to start a culture. The plant material can be obtained from existing cultures or mother plants. Cultures can be started from meristems, nodes, apical or axillary buds, leaf discs, pollen, seed embryos, or hypocotyl sections. Explants from mother plants must be surface sterilized before planting into liquid or on solid culture media. The type of explant and the composition of the culture media determine whether the explants grow into shoots, roots, or callus. Smaller explants are more likely to grow into disease-

free plants than larger ones. Microscopic meristems are best to eliminate viruses and viroids but technically difficult to prepare. Shoot development takes longer than in cultures started from buds or nodes.

Genotype

The genotype of a plant is the entire genetic information that is contained in the chromosomes and passed on through seeds. Cannabis is a diploid species. Its genome is contained in twenty chromosomes, ten each from the father and mother plant. The genome is the same in each cell except for pollen and egg cells that contain only one set of ten chromosomes. Location within the plant, chemical signaling, and environmental factors determine which genes of the genome are active and how a cell functions. Plants also contain genetic information in the chloroplasts and mitochondria that is passed on through the egg cells.

Gibberellin

Giberellins are a group of plant growth regulators that are synthesized in chloroplasts. They stimulate germination, cell elongation, and flower and trichome development. In Cannabis tissue culture they are used to produce longer internodes that help subculturing bushy shoots.

Hardening

The process of adapting shoots grown in tissue culture to greenhouse grow conditions. In culture the developing shoots rely on sugars in the culture media for their energy supply. Once leaves develop photosynthesis takes over. Temperature

and humidity in the culture vessels are stable and there is no exposure to pathogens or pests. Successful transfer to the greenhouse requires allowing the shoots to develop strong roots and photosynthesis. The best conditions for hardening are low to moderate light, stable, warm temperature, and high humidity. Keeping the shoots in domed trays until they have well developed roots and show new growth helps with the transition.

Hermaphroditism

This term is used in Cannabis when plant produce both male and female flowers. In strict botanical terms a hermaphrodite has flowers that contain both male and female parts. Hermaphroditism can be a result of stress or repeated cycles of creating feminized seeds. It can also occur spontaneously. Some strains are more prone to hermaphroditism than others. The gender of Cannabis plants is determined by the pair of sex chromosomes. XX plants are female, XY plants male. However, XX plants can be chemically induced to form male flowers, all of which produce gametes (egg and pollen cells) carrying the X chromosome. All female flowers that are pollinated with X pollen will produce XX-, or feminized seeds. Hermaphroditism can cause massive yield and quality losses when cultivation batches seed out.

Hop Latent Viroid (HLVd)

HLVd is a viroid pathogen that is widely distributed in hops, where is causes only minor symptoms. It consists of a 256 bases long, circular RNA. HLVd was first identified in Cannabis in California in 2019 and has since spread throughout the US

and Canadian Cannabis industry. Annual losses are estimated in the billions. Plants infected with HLVd produce lower yields and potency. Symptoms include stunted growth, brittle branches and stems, horizontal branching, and discolored and deformed leaves. Visible symptoms often appear only in flowering plants. HLVd infections spread when sap from an infected plant comes in contact with wounded tissue of another plant. HLVd can be stable outside plant cells (in water or on surfaces) and transmission through water has recently been shown. Insect transmission is likely but has not been experimentally proven. The most common transmission in Cannabis cultivation occurs through cutting tools and from mothers to clones. HLVd is readily transmitted through seeds and tissue culture unless the cultures are started from microscopic meristems.
HLVd can most reliably be detected through qPCR (quantitative reverse-transcription polymerase chain reaction). Early symptoms of HLVd can be absent or similar to other infections or over-watering. Maintaining an HLVd-free mother stock is critical for preventing crop losses.

Hyperhydricity

See Vitrification

Meristem

Meristems are microscopic clusters of non-differentiated cells that can develop into any type of tissue. Meristematic cells are like stem cells in humans. They are not specialized but depending on chemical (plant growth regulators) or environmental stimulation can become shoots, roots, leaves, or any other organ or tissue.

Meristems are located where cells divide, and plants grow. This includes shoot tips, axillary buds in the leaf axils, root tips, and the zone between phloem and xylem where stems and branches grow thicker. Shoot-tip and axillary meristems can be used in tissue culture as starting material.
Preparation of a meristem requires removing all surrounding tissues and is done under the microscope. Pure meristem culture is the best method to remove virus or viroid Infections but technically difficult and time consuming.
Cannabis tissue culture usually starts from cuttings containing one or more meristems. The term meristem culture in Cannabis is sometimes incorrectly used for cultures starting from small cuttings containing a bud.

Micro Propagation

See tissue culture.

Mold

The term mold is used for fungal growth that looks dusty or fuzzy (e.g., Botrytis bud mold and powdery mildew). Fungal growth that causes breakdown of tissue into slimy, soft matter is called rot (e.g. Fusarium root rot and crown rot). Many funguses form molds. Molds in tissue culture grow out from explants or start from spores in contaminated culture media. Surface sterilization of the explants cannot prevent mold that originates from endophytic fungi. Airborne fungus spores can also contaminate cultures, especially when the tissue culture lab is close to drying or trimming rooms.
Mold growth in tissue culture can be suppressed by antimicrobial media supplements. However, these cultures can

still produce plants with internal fungal infections carried over from the mother.

Mutation

Mutations are changes in the DNA sequence of a cell, such as deletion, addition, or replacement of single or multiple nucleotides. They happen through mistakes in the DNA replication process when cells divide or environmental factors such as radiation or exposure to mutagenic chemicals. Mutations in germline cells are passed on through seeds and clones. Mutations occur randomly at variable frequency. Cells can repair most mutations that only affect one of the two complimentary DNA strands. Mutations are not reversible and lead to stable changes in the phenotype of Cannabis strains over successive propagation cycles.

Phenotype

Phenotype is the sum of visible and measurable traits that are determined by the genotype and how the genes are regulated. Plants with the same genotype can have different phenotypes due to epigenetic changes, stress, and environmental factors.

Plant Growth Regulators

Plant growth regulators (PGRs), also called plant hormones are signal molecules that are produced within the plant and are transported through the vascular system. PGRs are commonly called plant hormones and control all aspects of plant development, including formation of shoots, roots, and leaves, flowering, and embryogenesis. PGR are effective at very low concentrations and finding the balance of PGRs in culture media is crucial for success. Synthetic analogs of natural PGRs are

frequently used in tissue culture. The most used PGRs in Cannabis culture are cytokinins, auxins, and gibberellins. Others are abscisic acid (stress response, seed and bud dormancy, leaf abscission), ethylene (fruit ripening, seed germination, senescence), jasmonic acid, and salicylic acid (both involved in plant resistance).

Plant Hormone

See Plant Growth Regulators.

Ploidy

Most species are diploids. They contain one set of chromosomes from each parent. Diploid Cannabis has twenty chromosomes. All cells are diploid except egg and pollen cells that contain one set of ten chromosomes each. Ploidy levels are given as lower-case n: n=haploid (single set of chromosomes), 2n=diploid (2 sets), 3n=triploid (3 sets), 4n=tetraploid. Higher ploidy levels are possible and used in some crops (e.g. strawberries, 8n) to increase fruit size and yield.

It is possible to double the number of chromosomes by treating dividing cells with chemicals that inhibit their separation after replicating the chromosomes. This results in creating tetraploid tissue from meristem cells or diploid germline (egg and pollen) cells. Triploid plants are the result of crossing tetraploid with diploid parents.

The results of creating tetraploid Cannabis have been variable. Stronger stems and broader leaves are visible traits, but yield increases have been minimal. Triploid Cannabis can have increased yield and is unable to produce seeds.

Somaclonal Variation

Somaclonal variation occurs when the genotype and phenotype of tissue culture plants grown from the same explant varies. The differences are due to mutations or re-arrangement of chromosomes, such as addition or deletion of DNA, or uneven distribution of chromosomes during cell division.

Several factors increase the likelihood of Somaclonal variation:

The type of explant. Plants regenerated from cultured tissue are genetically less stable than conventional clones. Shoots grown from callus are most susceptible to somaclonal variation.

The number of subcultures. Each cycle of subculturing adds to the frequency of genetic variation.

Imbalanced hormone composition in the culture media.

Chemical or environmental stress during culture.

Somatic Embryo

Plant embryos are formed during seed development and grow into seedlings after germination. They are composed of a small group of cells and have a typical bipolar structure. I some instances, embryos can develop from single cells that are not zygotes. These are called somatic embryos. In contrast to seed embryos, they are genetically identical to the tissue in which they were formed. The formation of multiple somatic embryos from callus grown in tissue culture is used for mass-propagation of many crop and ornamental plant species. However, there are few credible reports of somatic embryogenesis in Cannabis. Somatic embryogenesis is also a crucial step in genetic engineering of crops that are propagated through cloning.

Sterilization

Sterilization is a process of destroying microorganisms, viruses, viroids, and funguses on a surface or in a liquid. Tissue culture media and culture vessels are sterilized by autoclaving and tools by flaming. Starter explants for tissue culture are surface sterilized by submersing them in diluted bleach or in 70% alcohol. This is sufficient to remove surface borne contamination but not systemic pathogens that exist inside the plant tissue. Contamination in culture vessels often originates from within the explants.

Subculture

A subculture is started with explants from an existing culture. These explants do not have to be sterilized. Subcultures start from nodes of shoots grown in the original culture to multiply the number of clones, to replace exhausted media, or to transfer shoots to rooting media. Cultures can be maintained for extended time with repeated subculturing.

Tissue Culture

The plant term tissue culture is used for a wide variety of procedures where single cells, organs, or different types of cuttings are grown in a sterile environment. It involves the steps of preparing the cuttings (explants), surface sterilizing them, and transferring them into sterile liquid or solid culture media. The nutrients and growth regulators in the media determine whether the explants grow into shoots, roots, or callus. The common application in Cannabis is direct shoot regeneration from nodes or buds. These are collected from mother plants or existing cultures. Shoots developing in culture can branch and produce multiple buds that can be sub-cultured

or rooted. Many crop and ornamental plants that are propagated through tissue culture involve an initial step of growing explants into non-differentiated cell clusters (callus) which then are stimulated to produce large numbers of shoots from individual cells (somatic embryogenesis). This is not an established procedure for Cannabis and there are few credible reports of success.

Micro propagation is cloning in a sterile environment, starting with small cuttings. In contrast to conventional cloning that starts with shoot tips that are planted directly into rooting cubes or soil, micro propagation involves surface-sterilizing the cuttings and planting them into sterile culture media containing nutrients, sugar, and plant growth regulators. The developing shoots can then be transplanted to sterile rooting media or to rooting cubes. The process can involve a shoot multiplication step under culture conditions that requires one or more additional transplanting steps.

Transformation

Transformation is the process of controlled modification of the genome of a target organism. This involves constructing a DNA fragment (e.g. containing a gene) and introducing it into the target genome. This can be achieved through bacterial (e.g. Agrobacterium) or viral "vectors," or chemical or mechanical means. The recent discovery of the CRISPR-Cas9 method allows for introducing DNA constructs into specific locations of the target genome.

Transformation is used to add or disable properties of target plants, animals, bacteria, or fungi. Stable transformation

requires all that cells carry the modification. In plants this can be achieved through transforming a germ-line cell (pollen or egg cell) or recovering a plant from a single transformed cell (somatic embryogenesis). GMO (genetically modified organism) plants are offspring of transformed plants. Common traits include resistance to pests and pathogens, herbicide resistance, tolerance to environmental stress, enhanced yield and qualitative traits like color and nutritional content. Transformation can also be used to disable biochemical pathways. Recently Cannabis plants that cannot synthesize THCA have been created through Agrobacterium-mediated transformation.

Viroid

Viroids are a group of small plant pathogens that consist only of a circular RNA strand. Unlike viruses they have no protein coat. The different viroids that have been described so far have genomes of approximately 240-470 bases. In contrast to viruses, viroid genomes contain no readable genes. Within plants, viroids spread long-distance through the phloem like viruses. Cell to cell spread through plasmodesmata is much slower.

Virus

Viruses are submicroscopic pathogens that infect living cells of all kinds of organisms. They are entirely dependent on the host cell's metabolism to multiply. Viruses consist of a genome that can be single- or double- stranded DNA or RNA and a coat (capsid) composed of one or more proteins. Most plant viruses have single stranded RNA genomes that contain genes encoding

the capsid protein(s), a polymerase (enzyme that enables copying the genome), and sometimes proteins involved in cell-to-cell movement of the infection.
Virus infections are transmitted passively between plants through wounds (e.g., pruning shears), contaminated water, and insects or mites. Lacking their own metabolism viruses are not living beings and thus cannot be killed like fungal or bacterial pathogens. However, they can be destroyed by chlorine or peroxide-based disinfectants.
Cannabis viruses include Beet Curly Top Virus (BCTV), Lettuce Chlorosis Virus (LCV), Cannabis Cryptic Virus (CCV), and Tobacco Mosaic Virus (TMV). Symptoms of virus infection are often similar to other factors that can cause yellowing, leaf curling, or mosaic patterns.

Vitrification

The symptoms of vitrification include stunted growth, crooked leaves, and glassy and swollen tissue. Also called hyperhydricity, is a response to different stresses, mostly excessive humidity, and poor ventilation. Changing the agar and nutrient concentrations in the media and ensuring he culture vessels allow for air exchange can prevent vitrification. The term vitrification also describes the process of replacing cellular water with a cryoprotective liquid (e.g., glycerol) in preparation of long-term storage of tissue in liquid nitrogen.