# The Physiology of Aging in Cannabis Mother Plants: Implications for Longevity, Re-vegetation, and Flowering

## I. Introduction: The Aging Process in Plants

### A. General Principles of Plant Senescence: An Overview

Plant senescence represents the final, genetically programmed stage of development, characterized by a series of coordinated physiological and molecular changes that culminate in the death of cells, organs, or the entire plant. Far from being a passive decay, senescence is an active, highly regulated process crucial for plant fitness. It serves as an "altruistic death" mechanism, particularly in leaves, allowing for the systematic degradation of macromolecules and the remobilization of valuable nutrients (e.g., nitrogen, phosphorus, potassium) from senescing tissues to actively growing or storage organs, such as developing seeds, fruits, or younger leaves. This resource reallocation strategy has evolved to maximize the reproductive success and overall survival of the plant or its progeny.

The onset and progression of senescence are governed by a complex interplay of internal cues, such as developmental age and hormonal signals, and external environmental factors, including photoperiod, temperature extremes, nutrient availability, and various biotic and abiotic stresses. Physiologically, senescence is marked by a significant metabolic transition from anabolism (synthesis) to catabolism (breakdown). This involves the degradation of cellular structures, most notably chloroplasts, which leads to chlorophyll breakdown and the characteristic yellowing of leaves. Concurrently, proteins, lipids, and nucleic acids are dismantled to salvage essential nutrients.

At the molecular level, leaf senescence is orchestrated by a highly complex genetic program. This program is tightly controlled through multiple layers of regulation, encompassing chromatin remodeling, precise transcriptional control involving the upregulation of senescence-associated genes (SAGs) and downregulation of photosynthesis-related genes, as well as post-transcriptional, translational, and post-translational modifications. Transcription factors are key players in this process, integrating various signals and mediating widespread changes in gene expression. These general principles of plant senescence are fundamental to understanding the aging processes in *Cannabis sativa*, particularly in the context of mother plants maintained in a vegetative state for extended durations, where the dynamics of aging and senescence significantly influence their long-term viability and the quality of their clonal offspring.

### B. Relevance to Perennial and Clonally Propagated Species

In the plant kingdom, senescence manifests differently depending on life history. Annual plants, like wild cannabis, typically exhibit monocarpic senescence, where the entire plant senesces and dies after a single reproductive cycle, having allocated all resources to seed production. Perennial plants, however, may exhibit sequential senescence, where older leaves or plant parts senesce progressively while the plant continues to grow and can reproduce multiple times. Clonally propagated plants, such as cannabis mother plants, present a unique scenario. These plants are artificially maintained in a prolonged vegetative state to serve as a continuous source of cuttings. While this practice effectively postpones the whole-plant senescence associated with flowering and reproduction, it does not halt intrinsic cellular aging processes or the potential for localized senescence in older tissues. This extended vegetative phase introduces specific challenges, including the cumulative effects of environmental stress, pathogen accumulation, and potential genetic or epigenetic instability over time.

Modern cultivation techniques can extend the life of a cannabis mother plant, allowing favorable phenotypes to be perpetuated "almost indefinitely through cloning". This creates a scenario where the genetic line can be maintained for extended periods. However, the individual mother plant itself is not immune to the biological processes of aging. Numerous horticultural sources and practical observations indicate that mother plants eventually exhibit a decline in vigor, reduced cutting quality, and may require replacement after a certain period, typically ranging from months to a few years, although some exceptionally well-maintained individuals may last longer. This apparent contradiction highlights a crucial aspect of managing long-term mother stock: while the genotype can be preserved through serial cloning, the physiological performance of the individual mother plant is finite. This decline is not merely a function of increasing physical size but involves underlying physiological deterioration. Cultivation strategies must therefore address both the mitigation of age-related decline in the current mother plant and a systematic approach to mother stock rotation to ensure the consistent production of high-quality clones.

## II. Physiological and Molecular Aspects of Aging in Cannabis Mother Plants

### A. Cellular and Metabolic Changes in Long-Term Vegetative Cannabis

While dedicated research specifically tracking the detailed cellular and metabolic aging of cannabis mother plants over many years in a vegetative state is not abundant, significant insights can be drawn from general plant senescence principles and existing studies on cannabis physiology. As cannabis plants mature, even if maintained vegetatively, a gradual decline in overall physiological activity is anticipated. Studies on cannabis during its reproductive phase have noted lower stomatal conductance, photosynthesis, and transpiration rates in later stages of maturation, indicative of a natural decrease in metabolic efficiency. It is plausible that similar declines occur in very old mother plants.

Consistent with general plant senescence , aging cannabis tissues, even those not undergoing floral induction, likely experience a progressive shift from anabolic to catabolic processes. This could manifest as reduced vigor, slower growth rates, and a diminished capacity to produce healthy cuttings in older mother plants. Oxidative stress is a pervasive factor in biological aging across kingdoms. In plants, the accumulation of reactive oxygen species (ROS) can damage vital cellular components like proteins, lipids, and DNA if the plant's antioxidant defense systems become compromised with age or chronic stress. Although much of the research on cannabis and oxidative stress relates to the effects of its consumption on mammals , the fundamental mechanisms of ROS-induced damage are relevant to plant aging and could contribute to the physiological decline of long-term mother plants.

Furthermore, long-term mother plants, despite receiving consistent nutrient supplies, may develop subtle yet impactful nutrient imbalances or a state of "hidden hunger." This phenomenon could arise from several age-related factors: a decline in the efficiency of nutrient uptake by an aging root system, altered patterns of nutrient translocation within the plant, or the gradual depletion of specific micronutrients that are not always replenished adequately by standard vegetative feeding regimes over extended durations. General plant senescence involves significant nutrient remobilization. While mother plants are kept vegetative, older leaves still undergo localized senescence, and the efficiency of internal nutrient cycling might diminish. Cannabis plants are known to store photoassimilates and nitrogenous compounds in stem tissues during vegetative growth ; the capacity or efficiency of this storage and subsequent remobilization for new growth could decline with physiological age. Although mother plants are typically supplied with nitrogen-rich fertilizers to support vegetative growth , the complex interplay of nutrient uptake, assimilation, and internal allocation over several years might lead to specific deficiencies or antagonisms that are not immediately apparent as visible symptoms but contribute to a gradual loss of vigor and a decline in the quality of cuttings produced. Routine, comprehensive leaf tissue analysis could therefore be a valuable tool for detecting and correcting such imbalances in aging mother plants, potentially extending their productive lifespan.

### B. Hormonal Dysregulation in Aging Mother Plants (Auxins, Cytokinins, ABA, Ethylene, Gibberellins)

Phytohormones are critical regulators of plant growth, development, differentiation, and response to environmental stimuli, including the aging process. The balance and interplay among these signaling molecules are finely tuned, and disruptions or shifts in this balance are hallmarks of senescence. In long-term cannabis mother plants, progressive hormonal dysregulation is likely a key contributor to age-related decline.

**Table 1: Hormonal Dynamics and Their Impact in Aging Cannabis Mother Plants and Phase Transitions**

| Phytohormone | Primary Production Site(s) | Key Roles in Vegetative Growth & Health | Typical Changes/Imbalances in Chronically Vegetative/Aging Mothers | Impact on Mother Plant Vigor & Cutting Performance | Role & Desired Shifts During Re-vegetation Attempt | Role & Desired Shifts During Flowering Attempt of Aged Mother |
| --- | --- | --- | --- | --- | --- | --- |
| **Auxins** (e.g., IAA) | Apical buds, young leaves, developing seeds | Stimulate cell elongation, root formation (adventitious), apical dominance, vascular differentiation; delay leaf abscission and senescence | Potential decline in production or sensitivity; altered distribution; reduced auxin/cytokinin ratio in some contexts, or accumulation in older tissues leading to inhibition. | Reduced rooting of cuttings, decreased vigor, changes in branching patterns. | Increased auxin activity or sensitivity needed to promote new vegetative shoots and root development from dormant buds. | Auxin levels typically decrease to allow flowering; high auxin can inhibit flowering. |
| **Cytokinins** (e.g., Zeatin) | Root tips, developing embryos, fruits | Promote cell division, shoot and bud proliferation, lateral bud growth (counteracting apical dominance); delay senescence ("Richard Lang effect"). | Likely decline in synthesis or transport from aging roots; shift in auxin/cytokinin ratio favoring auxins or senescence promoters. | Reduced branching, slower growth, yellowing of older leaves (premature senescence), reduced cutting vigor. | Increased cytokinin levels or sensitivity needed to stimulate bud break and new shoot formation. | Cytokinin levels increase to promote new shoots and flowering tips during floral transition. |
| **Gibberellins** (GA) | Young leaves, developing seeds, roots, shoot apex | Promote stem and leaf elongation, cell division, seed germination, transition to flowering. | Active GA forms progressively degraded with leaf aging; potential overall decline in effective GAs. | Stunted growth, reduced internode elongation, possibly delayed response to stimuli. | May play a role in stimulating new shoot elongation if levels are restored. | Key for flower initiation and development (e.g., stretch). |
| **Abscisic Acid** (ABA) | Mature leaves, roots (especially under stress), seeds | Growth inhibitor; promotes dormancy, stomatal closure (stress response); accelerates senescence and abscission. | Levels may gradually increase with age or in response to accumulated chronic stress. | Reduced overall vigor, increased stress susceptibility, premature senescence of older leaves, potentially poorer cutting performance. | High ABA levels would antagonize re-vegetation; levels need to decrease. | ABA typically increases during later stages of flowering and senescence. |
| **Ethylene** | Most plant tissues, especially aging, stressed, or ripening tissues | Gaseous hormone; promotes fruit ripening, leaf and flower senescence, abscission; stress response. | Increased production or sensitivity in older, stressed tissues. | Accelerated aging of leaves, reduced shelf-life of cuttings, potentially reduced vigor. | High ethylene levels would promote senescence, hindering re-vegetation; levels need to be low. | Plays a role in flower maturation and senescence. |

*Data synthesized from:*

The transition to flowering in cannabis involves "profound hormonal changes" , where auxin production generally decreases, and levels of gibberellins (for stem elongation or "stretch") and cytokinins (promoting new shoots and floral primordia) increase. Conversely, senescence is characterized by a complex interplay where senescence-promoting hormones like ABA and ethylene become more dominant, while the influence of growth-sustaining hormones such as auxins, cytokinins, and gibberellins wanes. Exogenous application of hormones like GABA, ABA, and SA has been shown to influence cannabinoid biosynthesis genes and final cannabinoid content in cannabis, underscoring the plant's sensitivity to hormonal regulation.

Even if maintained in a strictly vegetative photoperiod, long-term mother plants are likely to undergo gradual, subtle shifts in their endogenous hormonal milieu. These might include a declining cytokinin-to-auxin ratio, or a slow increase in ABA levels or ethylene sensitivity, particularly in older tissues or those experiencing chronic low-level stress. Such shifts may not induce overt, plant-wide senescence but could "prime" the plant for a more rapid decline in vigor or a less robust physiological response when a major phase change, such as re-vegetation or flowering, is attempted. This physiological predisposition is influenced by factors like the age of the tissue within the plant, as explants from basal (more juvenile) portions of an aged mother plant can exhibit better regenerative capacity in vitro, partly due to differences in endogenous phytohormones. This suggests an age-related hormonal gradient or differential hormonal status within the aging mother plant itself. Consequently, attempts to induce phase changes in very old mother plants may face greater challenges due to this "primed" hormonal state, underscoring the importance of selecting cuttings from more juvenile parts if possible and managing stress meticulously.

### C. Genetic and Epigenetic Stability

The long-term maintenance of cannabis mother plants relies on their ability to faithfully replicate their genetic and phenotypic characteristics through clonal propagation. However, over extended periods and multiple generations of cuttings, questions arise regarding genetic and epigenetic stability.

**1. Somatic Mutations and Clonal Degradation:** Clonal propagation, by definition, aims to produce genetically identical copies of the mother plant. However, this ideal can be compromised by the accumulation of somatic mutations—genetic alterations occurring in the non-reproductive (somatic) cells of the mother plant during its extended vegetative growth or during the process of cloning itself. These mutations, if they occur in cells that give rise to new shoots used for cuttings, can be passed on to subsequent clonal generations.

The term "clonal degradation" or "clonal decay" (sometimes inaccurately referred to as "genetic drift" in this context ) describes the observed decline in vigor, yield, consistency of cannabinoid and terpene profiles, and overall performance of clones taken from very old mother plants or after many successive generations of cloning. This phenomenon is attributed, at least in part, to the accumulation of deleterious somatic mutations. The theory of Muller's Ratchet, which posits that asexual lineages tend to accumulate harmful mutations over time due to the absence of sexual recombination to purge them, provides a theoretical framework for understanding this potential decline in fitness. Research has shown that intra-plant genetic diversity exists within a single cannabis plant, with genetic differences increasing from the basal (bottom) to the apical (top) parts of the plant, suggesting mutations accumulate with growth and development.

Studies involving micropropagated cannabis have demonstrated a direct correlation between the number of subcultures (successive propagations in vitro) and the frequency of somatic mutations. For instance, one study identified thousands of polymorphic variants in cannabis clones, with a significant increase in these variants observed between the sixth and tenth subcultures. Critically, some of these mutations were found in genes crucial for plant development and, perhaps more directly relevant to cannabis cultivation, in genes involved in the cannabinoid and terpene biosynthesis pathways (e.g., *CMK*, *HDS*, *GPPS.ssu1*, *HMGS*, *PMK*, *MPDC*). Such mutations carry the potential to alter the plant's chemotype, affecting its medicinal or recreational properties. While some argue that clones are exact genetic replicas and that significant genetic change is unlikely , the evidence for somatic mutation accumulation, particularly from micropropagation studies, is growing. The concept of "regenerative mutation," where the process of tissue regeneration itself might introduce genetic changes, has also been proposed based on studies in other plant species.

**2. Epigenetic Drift and Its Implications:** Epigenetics refers to heritable changes in gene expression that occur without altering the underlying DNA sequence. These modifications, such as DNA methylation and histone modifications, can act as molecular switches, turning genes on or off in response to developmental cues, environmental stimuli, stress, and aging.

In long-term mother plants, epigenetic drift—the gradual accumulation of random or stress-induced epigenetic changes—can lead to alterations in gene expression patterns over time. This can affect various phenotypic traits, including vigor, stress response, and potentially secondary metabolite production, even if the underlying genetic code remains largely unchanged. Environmental conditions in the grow room, nutrient status, and the stress of repeated pruning can all contribute to epigenetic modifications. While much of the research on cannabis and epigenetics has focused on the effects of cannabis consumption on human or animal epigenomes , these studies demonstrate that cannabis-derived compounds can interact with and modify epigenetic machinery, highlighting the plant's own susceptibility to such regulation.

In plant systems, epigenetic variations can be environmentally induced or arise spontaneously, and their stability can vary. Asexual reproduction, by bypassing the meiotic resetting of some epigenetic marks that occurs in sexual reproduction, might allow for more stable transmission of certain epigenetic states across clonal generations. Studies on in vitro cannabis propagation have suggested that phenomena like declining multiplication rates in apical explants over successive subcultures could be linked to epigenetic mechanisms.

**3. Telomere Dynamics in Clonal Aging:** Telomeres are repetitive DNA sequences at the ends of chromosomes that protect them from degradation and fusion, playing a crucial role in maintaining genome stability. With each cell division, telomeres can shorten, and critically short telomeres are associated with cellular senescence (the cessation of cell division) and organismal aging. Stress, including oxidative stress and environmental hardships, can accelerate telomere attrition.

In the context of clonal aging in cannabis, telomere dynamics could play a role. If telomeres shorten progressively in the somatic cells of a mother plant over its extended lifespan and through numerous cell divisions required for growth and cutting production, this could contribute to a decline in cellular function and overall plant vigor. Cuttings taken from such a plant would inherit cells with already shortened telomeres, potentially leading to earlier senescence or reduced proliferative capacity in the resulting clones. Some research suggests that clonally maintained crop species, including hemp, tend to have long telomeres, possibly as an adaptation requiring long-term chromosomal stability. However, the stress of long-term cultivation and repeated pruning might still impact telomere integrity. Cloning from older mother plants, especially those that have undergone stressful flowering cycles (if re-vegetated), is considered suboptimal, partly due to compromised genetic integrity which could involve telomere damage. While direct research on telomere length and clonal aging specifically in cannabis mother plants is limited, the general principles of telomere biology suggest it as a potential contributing factor to age-related decline.

The decline observed in long-term mother plants and their subsequent clones is likely a multifaceted issue, stemming not from a single isolated cause but from a synergistic interplay of these genetic, epigenetic, and physiological factors. Somatic mutations accumulate with cell division and age ; epigenetic patterns can drift due to chronic stress or developmental changes, altering gene expression ; and, as will be discussed further, pathogen load tends to increase over time, imposing additional stress. These elements can create a reinforcing cycle: for example, pathogen infection is a significant stressor that can induce epigenetic changes and potentially weaken the plant's repair mechanisms, possibly leading to an increased rate of somatic mutation or reduced ability to cope with existing mutations. This "aging triangle" underscores the need for a holistic management approach that addresses genetic integrity, minimizes stress, and actively controls pathogen presence.

### D. Decline in Vigor and Rooting Potential of Cuttings from Aged Mothers

A common observation among cultivators is that cuttings taken from older cannabis mother plants often exhibit reduced vigor, slower rooting times, and lower overall rooting success rates compared to cuttings from younger, more vigorous mothers. This decline is a significant concern as it directly impacts propagation efficiency and the quality of new plant stock.

Research supports these observations. Studies in other plant species, and some in cannabis, indicate that juvenile plant material generally possesses superior rooting capabilities compared to mature material. This is often attributed to higher endogenous levels of auxins and other rooting co-factors in juvenile tissues. As mother plants age, the physiological state of their tissues changes. Cannabis producers have noted that cuttings from mother plants can become less vigorous and may show altered cannabinoid levels over time. A study on 2.5-year-old cannabis mother plants demonstrated that the source of the explant (cutting) significantly influenced its performance in vitro; explants taken from the basal (lower) portions of the mother plant exhibited better multiplication rates and morphological characteristics indicative of juvenility (e.g., shorter, wider leaves) compared to explants taken from the apical (upper) regions. Apical explants, in contrast, showed a decline in multiplication capacity over successive subcultures.

The underlying reasons for this decline in rooting potential and vigor are multifaceted. Hormonal imbalances within the aging mother plant are likely a primary contributor. A reduction in the production or transport of auxins, or an unfavorable shift in the auxin/cytokinin ratio, can impair adventitious root formation. The general health of the mother plant also plays a crucial role; accumulated stress, potential nutrient deficiencies or imbalances, and a higher pathogen load in older mothers can all negatively affect the vitality and rooting capacity of the cuttings derived from them. The age and health of the mother plant are explicitly mentioned as factors affecting rooting quality and success in cannabis.

Interestingly, the aging process may not be uniform throughout the entire mother plant. The superior performance of basal explants from aged cannabis mothers suggests that different parts of a single plant may exhibit varying degrees of "physiological age" or "juvenility." While chronologically older, basal tissues might be developmentally less committed or retain a more favorable hormonal and metabolic state for regeneration compared to the more actively growing but also potentially more stressed or mutated apical regions. This implies a gradient of aging within the mother plant itself. Therefore, when propagating from older, valuable mother stock, selecting cutting material from more basal, yet still vigorous, shoots might yield more successful outcomes than relying exclusively on apical cuttings, which could be more affected by accumulated age-related detriments. This observation also lends support to practices like renewing mother plants from clones taken from their more juvenile basal sections.

### E. Pathogen Accumulation and Immune Response in Older Plants

Long-term cannabis mother plants, due to their extended lifespan and the common practice of repeatedly taking cuttings, are particularly vulnerable to the accumulation of systemic pathogens. These pathogens can include viruses, viroids (such as the economically significant Hop Latent Viroid, HLVd), fungi, and bacteria. Each act of taking a cutting creates a wound, providing an entry point for these opportunistic organisms.

Over time, this pathogen load can build up, often asymptomatically at first, leading to a gradual decline in plant vigor, slower growth rates, reduced yield potential of subsequent clones, and diminished overall quality. Specific diseases like Fusarium root and crown rot, Pythium root rot, and powdery mildew are common concerns in cannabis cultivation, and mother plants serve as potential reservoirs. Older mother plants may also exhibit a weakened immune response or be under a state of chronic physiological stress, making them more susceptible to infection and less capable of effectively combating pathogens.

The impact of these pathogens, especially systemic ones like HLVd, can be profound. HLVd, for instance, is known to cause stunting, reduced trichome production, and lower cannabinoid and terpene yields, symptoms that can easily be mistaken for general age-related decline or "clonal degradation". This highlights a critical consideration: many observed instances of declining vigor and performance attributed solely to the age or genetic drift of mother plants might, in reality, be significantly driven by a progressive and often undetected accumulation of pathogens. The restoration of vigor observed when infected cultivars are "cleaned" through techniques like meristem tissue culture, which can eliminate systemic pathogens , strongly supports the idea that pathogen load is a major contributor to the diminished performance of long-term mother stock. Therefore, attributing all decline merely to "old age" or "genetic wear-out" may overlook this crucial, and often manageable, factor. Rigorous pathogen screening, strict sanitation, and the periodic use of pathogen-remediation techniques like tissue culture are paramount for extending the productive lifespan of mother plants and ensuring the health and quality of their clonal offspring.

### F. Potential Biomarkers of Vegetative Senescence in Cannabis

Identifying reliable biomarkers for vegetative senescence in cannabis mother plants maintained for extended periods is an area requiring more dedicated research. However, based on general principles of plant aging and existing cannabis studies, several potential physiological and molecular indicators can be proposed.

**Physiological Markers:** A decline in photosynthetic efficiency, measurable through parameters like \text{CO}\_2 assimilation rates or chlorophyll fluorescence, could indicate reduced metabolic activity characteristic of aging. Reduced transpiration rates and stomatal conductance may also be observed. Visible signs such as gradual yellowing or necrosis of older, lower leaves, even under optimal nutrition, point towards localized senescence. A consistent decline in the rooting success rate, rooting speed, or initial vigor of cuttings taken from a mother plant over time is a strong practical indicator of diminishing maternal health. Leaf nutrient analysis might reveal altered uptake or mobilization patterns, as nutrient depletion or imbalance can trigger or accelerate senescence.

**Molecular Markers:** The expression levels of Senescence-Associated Genes (SAGs), well-characterized in model plants , could serve as molecular indicators if homologous genes are identified and validated in cannabis. Changes in the endogenous balance of key phytohormones are central to senescence. Monitoring the ratios of cytokinins to auxins (a decrease might indicate aging) or absolute levels of ABA and ethylene (or their biosynthetic pathway genes and precursors) could provide insights. Markers of oxidative stress, such as increased levels of reactive oxygen species (ROS) or upregulated activity of antioxidant enzymes, might reflect an age-related increase in cellular stress.

While more complex to implement routinely, tracking the frequency of somatic mutations in specific developmental or metabolic genes could offer a genetic marker of accumulated aging. Similarly, alterations in DNA methylation patterns at specific genomic loci, indicative of epigenetic drift, could be monitored.

Changes in the secondary metabolite profile of vegetative tissues might also hold potential. Notably, Cannabichromenic Acid (CBCA) concentrations have been reported to be higher in young cannabis plants, declining as the plant matures. This observation suggests that a progressive decrease in CBCA levels in the leaf tissue of vegetatively maintained mother plants over extended periods could serve as a non-invasive biochemical marker of physiological aging. Such a decline in a compound associated with juvenility might signal an internal shift towards a more mature physiological state, potentially preceding more overt symptoms like a significant loss of cutting vigor. While THC and CBD are primarily associated with flowering tissues , subtle changes in minor cannabinoids or even specific terpenes in vegetative parts over long durations might also prove indicative, though this requires further investigation. Monitoring CBCA could offer an early warning for cultivators, aiding in decisions regarding mother stock rotation before a substantial decline in clone quality becomes apparent.

## III. Maintaining Health and Productivity in Long-Term Cannabis Mother Plants

Ensuring the sustained health and productivity of cannabis mother plants over extended periods is a cornerstone of efficient and consistent clonal propagation. This requires a multifaceted approach encompassing meticulous nutritional management, environmental optimization, strategic pruning, diligent root care, and robust pathogen control.

### A. Nutritional Management Strategies for Aging Plants

A tailored nutritional program is vital for mother plants, which are perpetually maintained in the vegetative state. This phase demands a higher proportion of Nitrogen (N) to support vigorous foliage and stem development, compared to Phosphorus (P) and Potassium (K). While N fuels green growth, P is crucial for healthy root development and energy transfer, and K contributes to overall plant health, enzyme activation, and stress resistance. Adequate levels of essential micronutrients (e.g., iron, zinc, manganese) and secondary macronutrients like calcium and magnesium (often supplied via Cal-Mag supplements) are also critical to prevent deficiencies, support myriad metabolic processes, and maintain hormonal balance.

It is important to avoid excessive feeding, as this can lead to nutrient antagonisms, salt buildup in the medium, nutrient lockout, and physiological stress, paradoxically causing deficiency symptoms or reduced vigor. Regular monitoring of the root zone for nutrient accumulation is advisable. As mother plants age, their nutrient requirements may subtly change , potentially necessitating adjustments to the standard vegetative feed. Research indicates that while cannabis can tolerate high nutrient concentrations, this doesn't always translate to improved yield or quality, and luxury uptake (absorption beyond immediate needs) can occur. For instance, optimal nitrogen supply for vegetative cannabis has been identified around 160 mg/L, with lower levels causing deficiencies and significantly higher levels leading to developmental restrictions, possibly due to ion toxicity or induced limitations in carbon fixation. The use of enzymatic products can aid in breaking down accumulated salt residues in the growing medium, thereby improving nutrient availability. Employing organic fertilizers can also foster a healthy soil microbiome, which contributes to nutrient cycling and root health.

### B. Optimizing Environmental Conditions (Light, Temperature, Humidity, Airflow)

Creating and maintaining an optimal growing environment is paramount for the longevity and productivity of mother plants. **Light:** A consistent photoperiod of 18 to 24 hours of light per day is essential to keep mother plants in a continuous vegetative state and prevent the initiation of flowering. Any inconsistency in light hours can induce stress. Lights with a blue-dominant spectrum (typically 4000K–6500K), such as full-spectrum LEDs or T5 fluorescent lights, are preferred for vegetative growth as they promote compact, bushy plants with strong stems. **Temperature:** The ideal ambient temperature range for cannabis mother plants is generally between 22–26°C (approximately 70-80°F). Temperatures significantly above 31°C or below 15.5°C can impede growth and potentially affect plant health and potency development. **Humidity:** Relative humidity levels of 60–70% are often recommended for the early vegetative stage, with a slight reduction possible as the plant matures. Persistently high humidity, especially around the root ball, can contribute to fungal issues and impair root health. **Air Circulation and Ventilation:** Robust air circulation and regular air exchange are critical. Good airflow helps to prevent the establishment of pests and diseases (like powdery mildew ), avoids the creation of stagnant microclimates, ensures uniform temperature and humidity distribution, and facilitates gas exchange (\text{CO}\_2 uptake and \text{O}\_2 release) at the leaf surface. The grow space itself should be kept meticulously clean, lightproof (to prevent unintended flowering cues), and well-sealed to maintain environmental control.

### C. Pruning and Training Techniques to Mitigate Aging Effects

Strategic pruning and training are indispensable for managing mother plant architecture, promoting continuous production of high-quality cuttings, and mitigating some effects of aging. Regular pruning helps maintain a desirable shape (typically short and bushy), encourages the development of multiple lateral branches (providing more sites for cuttings), allows for the removal of old, damaged, or non-productive growth, and improves light penetration and air circulation within the canopy.

Techniques such as "topping" (removing the apical meristem or main growing tip) and "FIMing" (a modified topping technique) are commonly used to break apical dominance, thereby stimulating the growth of lower lateral branches and creating a more multi-branched structure. The removal of apical stems reduces the downward flow of auxin, which normally suppresses lateral bud growth, allowing cytokinins (transported from the roots) to promote the outgrowth of these buds. While pruning is beneficial, excessive defoliation (removal of too many leaves) should be avoided, as leaves are essential for photosynthesis and energy production, and their over-removal can slow growth and limit the plant's capacity to produce new cuttings. Strategic pruning can also prevent the lower portions of the plant from becoming overly woody and unproductive, ensuring a continuous supply of fresh, viable cutting material.

Beyond continuous light pruning for cutting harvest and shaping, a more aggressive, periodic "rejuvenative pruning" might offer benefits for very long-term mother plants. This approach, which involves a more significant reduction of the plant's biomass while carefully preserving healthy basal or younger shoots, could more effectively reset the plant's architecture and hormonal balance. Such a practice could mimic how some perennial shrubs respond to heavy browsing or seasonal dieback, forcing vigorous new growth from more juvenile tissues at the base of the plant. This type of pruning could lead to a more substantial shift in the auxin/cytokinin balance, potentially delaying certain aspects of physiological aging more effectively than continuous, less intensive pruning. However, this must be carefully balanced against the immediate and ongoing need for cuttings in a production environment.

### D. Root Health Management and Re-potting Strategies

The health and vitality of the root system are fundamental to the overall health, longevity, and productivity of a cannabis mother plant. A well-developed and healthy root mass is essential for efficient water and nutrient uptake, anchorage, and the synthesis of crucial plant hormones like cytokinins.

Choosing an appropriate growing medium that offers good drainage and aeration, such as high-quality potting soil, coco coir, or soilless mixes, is critical to prevent waterlogged conditions that can lead to oxygen deprivation and root diseases like Pythium root rot. The pot size should be adequate to allow for steady root development without being excessively large, which might encourage too rapid top growth or inefficient medium utilization; containers in the 10–15 liter range are often cited as suitable for compact mother plant care.

Over extended periods, mother plants can become "root-bound," where the roots densely fill the container and begin to circle along the pot walls. This condition restricts further root growth, impairs nutrient and water uptake, and can lead to plant stress and reduced vigor. To alleviate this, periodic root pruning is recommended. This practice involves carefully removing the plant from its pot, inspecting the root ball, and trimming away a portion (e.g., the outer 1/3 to 1/2) of the circling or overly dense roots using clean, sharp tools. The plant is then typically repotted into the same or a slightly larger container with fresh growing medium. Root pruning encourages the growth of new, fine feeder roots, which are more efficient at absorption, and helps maintain a healthy root-to-shoot ratio. Re-potting into fresh medium also replenishes nutrients and improves soil structure.

Root pruning is more than just a physical management technique; it can be a key intervention to reset nutrient uptake dynamics and favorably influence root-to-shoot hormonal signaling. Cytokinins, which play a vital role in delaying senescence and promoting shoot growth, are primarily synthesized in active root tips. By stimulating the formation of new, vigorous root tips, root pruning may enhance cytokinin production and its transport to the aerial parts of the plant, thereby helping to mitigate some physiological aging effects. Thus, proactive and regular root pruning should be considered an integral part of long-term mother plant care, potentially offering benefits to hormonal balance and nutrient cycling that extend beyond simply relieving physical root constraint.

### E. Pathogen and Pest Management

Vigilant pathogen and pest management is crucial for maintaining the health and extending the productive lifespan of cannabis mother plants, which can act as reservoirs for various detrimental organisms if not properly managed. Regular and thorough inspection of plants for early signs of common pests (e.g., spider mites, aphids, thrips, fungus gnats) and diseases (e.g., powdery mildew, Botrytis, root rot, viroids) is the first line of defense.

Creating an environment that is inhospitable to pathogens and pests is key. This includes maintaining optimal air circulation and appropriate humidity levels, and avoiding overwatering, all of which help to minimize conditions favorable for fungal growth and certain insect proliferation. Strict sanitation protocols are non-negotiable. This involves routinely cleaning and disinfecting the grow space, tools (ideally using dedicated tools for each mother plant or sterilizing between plants to prevent cross-contamination of diseases like HLVd), pots, and any equipment used in the mother room.

Preventative strategies can include the use of beneficial insects, biological control agents, or soft pesticides like neem oil or potassium bicarbonate, particularly as mother plants are not intended for direct consumption of flowers. It is critical that any stock plants used to establish mothers are tested and confirmed to be pathogen-free. Any new plants or clones brought into the facility should be quarantined before being introduced into the mother plant area to prevent the introduction of new pests or diseases.

For valuable genetic lines that may have accumulated pathogens over time, tissue culture techniques, specifically meristem culture, offer a powerful method for pathogen eradication, effectively "cleaning" the stock. Given the risk of pathogen buildup, a systematic mother plant turnover or replacement schedule (e.g., every 3-12 months) is a common and recommended practice in commercial cultivation to ensure a continual supply of healthy, vigorous clones and to break potential disease cycles.

## IV. Re-vegetation of Mature and Aged Cannabis Mother Plants

Re-vegetation, or "re-vegging," is the process of inducing a cannabis plant that has undergone flowering to revert to a state of vegetative growth. This technique can be employed to preserve genetics if clones were not taken prior to flowering, or to obtain multiple harvests from a single plant.

### A. Physiological Mechanisms and Hormonal Shifts During Re-vegetation

The primary physiological trigger for re-vegetation is a significant change in the photoperiod. Cannabis is typically a short-day plant, meaning it initiates flowering when the daily period of darkness increases to a critical length (commonly 12 hours). To induce re-vegetation, the photoperiod is switched back to a long-day regime, usually providing 18 to 24 hours of light per day. This extended light exposure inhibits the production and action of flowering-promoting hormones (florigen) and signals the plant to resume vegetative development.

The hormonal balance within the plant undergoes a significant shift. During the transition to flowering, auxin production typically decreases while levels of gibberellins (associated with stem elongation or "stretch" at the onset of flowering) and certain cytokinins (involved in promoting new shoot and flower primordia) increase. Re-vegetation requires a reversal of this state, favoring hormones that promote vegetative growth. This likely involves an increase in auxin activity relative to flowering promoters, and a cytokinin profile that supports the development of vegetative shoots and leaves rather than floral structures. Nitrogen-rich fertilizers are reintroduced to provide the necessary building blocks for new leaf and stem production. The plant experiences considerable physiological stress during this transition from a reproductive to a vegetative state. Initial new growth following re-vegetation can often appear abnormal, with leaves that are smooth-edged and single-bladed, before the plant gradually resumes its typical vegetative morphology.

### B. Common Techniques and Best Practices

Successful re-vegetation hinges on several key practices. The most critical is the **light cycle adjustment**, shifting from a 12/12 light/dark cycle to a long-day photoperiod of 18/6, or even 24/0 (continuous light) for the initial 2-3 weeks to strongly promote vegetative growth.

**Selective harvesting and pruning** at the time of the initial flower harvest are also vital. It is crucial to leave some plant material, particularly lower buds, shoots, and leaves, on the plant. These remaining structures contain dormant meristems that will give rise to the new vegetative growth. A common approach is to harvest the upper one-third of the plant (main colas), selectively trim the middle third to remove larger flowers while retaining fan leaves, and leave the lower one-third largely intact, removing only the largest terminal flowers. New vegetative growth often emerges more rapidly from these lower branches.

**Nutrient regimes** must be switched back from flowering formulas (typically higher in P and K) to nitrogen-rich vegetative feeds to support the development of new leaves and stems. Some cultivators administer a strong dose of nitrogen immediately post-harvest to provide a clear signal to the plant to recommence vegetative growth.

Maintaining **optimal environmental conditions** for vegetative growth (appropriate temperature and humidity) is also important. Patience is required, as the transition back to vigorous vegetative growth can be slow, often taking several weeks for new, healthy shoots to become well-established. Optional enhancements include transplanting the re-vegged plant into a larger container with fresh soil to encourage new root growth and overall vigor.

### C. Challenges Associated with Re-vegetating Older Specimens

Attempting to re-vegetate older or long-term mother plants presents distinct challenges compared to re-vegetating younger plants that have only completed a single flowering cycle. Older plants are generally more susceptible to the physiological stress inherent in the re-vegetation process, which can impact their recovery speed and overall health. Their inherent vigor is often already diminished due to age , which can translate to slower re-vegetation, less robust new growth, and potentially lower success rates. Signs of stress in regenerated plants, such as a reduced number of leaflets per leaf, shriveled leaves, and generally slow growth, can be more pronounced in older individuals.

Furthermore, aged mother plants may carry a higher burden of accumulated issues, such as a significant pathogen load or a greater number of somatic mutations and unfavorable epigenetic changes. These pre-existing conditions can hinder successful re-vegetation or compromise the quality and health of the rejuvenated plant. The established hormonal balance of a mature, potentially senescing plant might also exhibit greater "inertia," making it more resistant to shifting back to a strong vegetative drive.

The success of re-vegetation is not solely dependent on the chronological age of the mother plant but is more critically linked to its "physiological age"—a composite of its genetic integrity, epigenetic status, accumulated pathogen load, and overall stress history. Mother plants that are already showing significant signs of decline (e.g., poor cutting performance, increased disease susceptibility, markedly reduced vigor) are unlikely to re-vegetate successfully or produce a vigorous, productive plant post-re-veg. Re-vegetation is therefore more viable as a strategy to extend the utility of relatively healthy, albeit chronologically older, mother plants rather than a "rescue" mission for individuals already in advanced decline. A thorough assessment of the mother plant's overall health status is crucial before attempting re-vegetation. While specific success rates for re-vegetating aged mother plants are not well-quantified in available literature, the general indication is that outcomes become less predictable and potentially less favorable with increasing physiological age and accumulated stress.

### D. Expected Outcomes: Vigor, Growth Patterns, and Subsequent Clone Performance

The outcomes of re-vegetating a mature cannabis plant can vary. Initially, the new vegetative growth emerging from the remaining buds and nodes can be atypical. It's common to see the plant produce smooth-edged, single-bladed leaves before transitioning back to the more familiar serrated, multi-fingered leaf morphology. Once re-established, the plant often develops a very bushy structure with numerous new growth sites, a result of many dormant axillary buds being activated.

The vigor of the re-vegetated plant can be a mixed bag. On one hand, the plant benefits from an already established and often extensive root system, which can save considerable time compared to starting from seed or a small clone. Some reports suggest that plants with mature root systems can re-veg more quickly and potentially yield more in subsequent harvests. However, if the original mother plant was very old, stressed, or had diminished vigor, the re-vegetated plant may also lack robustness and grow more slowly than a plant propagated from a fresh seed or a clone from a young, prime mother.

Clones taken from a successfully re-vegetated plant will be genetically identical to the original mother plant, preserving its unique characteristics. However, the physiological state of the re-vegetated mother at the time of cutting can influence the initial performance of these clones. If the re-vegetated mother experienced significant stress or its overall health was compromised during the process, cuttings taken from it might exhibit slower rooting or reduced initial vigor. The source of tissue within an aged plant has been shown to impact in vitro performance, underscoring that the physiological condition of the propagule source is key. If the re-vegetated plant is subsequently flowered again, yields may be lower than the initial harvest, and there are anecdotal reports of potential declines in quality (potency or flavor), although some suggest these can be mitigated or even improved with specific techniques.

### E. Success Rates and Factors Influencing Rejuvenation

Specific, quantified success rates for the re-vegetation of aged cannabis mother plants are not extensively documented in scientific literature. Success is highly variable and contingent upon several factors, including the genetic predisposition of the strain, the overall health and physiological age of the mother plant, and the precision of the techniques employed.

Factors that generally favor successful re-vegetation include starting with a mother plant that, despite its age, is still relatively healthy and vigorous. Certain cannabis strains may possess more robust genetics that lend themselves better to regeneration. Meticulous application of re-vegetation techniques, such as providing the correct long-day photoperiod, appropriate nitrogen-rich nutrition, optimal environmental conditions, and careful pruning that leaves sufficient foliage and small buds to initiate new growth, is critical.

Conversely, factors that can hinder success include extreme age or poor pre-existing health of the mother plant, excessive stress imposed during the re-vegetation process (e.g., improper handling, extreme environmental fluctuations), and inadequate nutrient or light management. Some strains may inherently be more recalcitrant or "resistant" to re-vegetation, although the term "resistant" in regarding pure Indicas likely implies resilience and adaptability, making them potentially *better* candidates for re-vegetation. In vitro studies have shown that even within a single aged (2.5-year-old) mother plant, explants from basal regions exhibit greater rejuvenation capacity compared to apical regions, suggesting that the source of the regenerative tissue within an old plant is a critical determinant of success.

## V. Flowering of Aged Cannabis Mother Plants

Inducing flowering in cannabis mother plants that have been maintained in a vegetative state for extended periods, often years, is a practice considered by cultivators for various reasons, such as assessing the plant's ultimate floral traits before retiring the line, or attempting a final harvest from valuable genetics.

### A. Feasibility and Challenges of Inducing Flowering in Senescent or Long-Term Vegetative Mothers

It is physiologically feasible to induce flowering in cannabis plants that have been kept in a prolonged vegetative state. The transition is typically triggered by shifting the photoperiod from a long-day (e.g., >16 hours of light) to a short-day regime (usually 12 hours of light and 12 hours of dark).

However, flowering very old or potentially senescent mother plants presents several challenges:

* **Reduced Vigor:** Aged plants often exhibit diminished physiological vigor. Flowering is an energy-demanding process, and an older plant may lack the reserves or metabolic capacity to support robust flower development and maturation. Wild cannabis, an annual, typically expends all its stored energy on flowering and seed production, after which it senesces and dies.
* **Hormonal Imbalance/Inertia:** A mother plant maintained in a vegetative state for years develops a hormonal profile strongly favoring vegetative growth. Shifting this entrenched hormonal balance to one conducive to flowering might be slower or less efficient compared to younger plants. Various factors such as poor nutrition, suboptimal temperatures, drought, salt buildup in the medium, exposure to certain exogenous chemicals, or pathogen presence can negatively affect or delay flowering ; these issues may be more prevalent or have cumulative effects in aged mother plants.
* **Nutrient Requirements:** The nutritional needs of cannabis shift dramatically from the vegetative to the flowering stage, with an increased demand for phosphorus and potassium and a decreased need for nitrogen. An aged mother plant, potentially with a less efficient root system or altered nutrient uptake capabilities, might struggle to adapt to these changing demands.
* **Plant Architecture:** Long-term mother plants can become very large, with extensive woody tissue and a dense canopy. This architecture may not be optimal for flower production, leading to issues with light penetration to lower bud sites and poor air circulation, which can affect bud quality and increase disease risk. Strategic pruning prior to inducing flowering is essential.
* **Stress Susceptibility:** The transition to flowering represents a significant physiological stress. Older plants, potentially already under some degree of chronic stress, may be more susceptible to further complications during this phase.

Cannabis mother plants kept in a vegetative state for exceptionally long durations (e.g., multiple years) might enter a condition that could be described as "vegetative stagnation." In this state, the cumulative effects of cellular aging, accumulated epigenetic modifications, and an entrenched hormonal profile strongly biased towards vegetative functions may impede a swift, efficient, and productive transition to the flowering phase. Such plants might exhibit a delayed floral initiation, a protracted flowering period, or less than optimal bud development, even if they possess a large vegetative biomass. The inherent energy reserves and reproductive capacity, which are finite even in cultivated varieties , may be significantly diminished in these over-aged individuals. Therefore, while flowering is possible, expectations for yield and quality from extremely old mother plants should be tempered, and pre-flowering health optimization becomes even more critical.

### B. Impact of Prolonged Vegetative Stage on Floral Development

The duration of the vegetative stage significantly influences the final size and structure of the cannabis plant when it enters flowering. Plants vegetated for longer periods can achieve substantial biomass, potentially offering more budding sites if the plant remains healthy and vigorous. However, the characteristic "stretch" that occurs after the switch to a 12/12 photoperiod (where Indica varieties may double in size, and Sativa-dominant strains can stretch 2-3 times or more ) means that an already large mother plant could become unmanageably tall or expansive in a typical flowering room.

The actual duration of the flowering period itself (from photoperiod change to harvest readiness) is primarily determined by the strain's genetics. However, the physiological age and health of the plant could potentially influence the timing of maturation or the uniformity of ripening. Some research suggests that the standard 12L:12D photoperiod may not be optimal for all cannabis lines, with some cultivars benefiting from slightly longer light periods during flowering in terms of yield, though this can sometimes negatively impact THC concentrations.

Energy allocation is a critical factor. An older plant, even if large, might struggle to adequately supply nutrients and photosynthates to a vast number of developing bud sites. This could result in a higher proportion of smaller, less dense "popcorn" buds, particularly in the lower and more shaded areas of the canopy. Strategic pruning to remove lower, unproductive growth before initiating flowering is often recommended to concentrate the plant's energy into the upper, more light-exposed colas. Studies on younger plants have shown that extending the vegetative phase can lead to increased inflorescence yield, primarily due to a higher number of inflorescences rather than larger individual ones. How this translates to multi-year-old mothers, with potentially different energy dynamics, is less clear.

### C. Expected Outcomes:

**1. Flower Yield and Morphology:** The potential flower yield from an aged mother plant is complex. A larger plant inherently has more potential budding sites, which could translate to a higher overall yield if the plant is healthy and vigorous enough to support their development. However, if the plant is physiologically exhausted or stressed, it may produce a meager harvest or even fail to complete the flowering cycle effectively. Reports from cultivators who have re-vegged and re-flowered plants multiple times suggest that subsequent harvests can be substantial, but this refers to plants that have already flowered at least once, not necessarily a first flowering from a very old, continuously vegetative mother.

Harvesting too early is a common mistake that significantly reduces yield and compromises quality ; this is a general principle but particularly relevant if aged plants exhibit altered maturation timelines. Bud morphology, including density and size, will be heavily influenced by the plant's ability to efficiently translocate resources to the developing flowers. Light penetration and canopy position are critical; studies show that inflorescences from lower canopy positions tend to have reduced cannabinoid concentrations. This would be a major consideration for large, dense, aged mother plants.

**2. Cannabinoid and Terpene Profile Stability and Potency:** Genetically, clones taken from a mother plant are expected to have the same chemotypic potential as the mother. However, the actual expression of cannabinoids and terpenes is highly influenced by environmental conditions during growth and flowering, as well as the physiological health and maturity of the plant.

If an aged mother plant is stressed, has accumulated pathogens, or its metabolic efficiency has declined, the resulting flowers might exhibit altered cannabinoid and terpene profiles or lower overall potency compared to flowers from the same genetic line harvested in its prime. For example, inadequate nutrient supply or stress from drought can reduce secondary metabolite concentrations. Conversely, optimal light intensity during flowering has been shown to increase cannabinoid levels. The timing of harvest is crucial, as cannabinoid and terpene profiles evolve throughout the flowering period and into senescence. For instance, CBCA is known to decline with maturation , while THC content in a normal flowering cycle generally increases up to the budding stage, plateaus, and then may decline with senescence. How these dynamics play out in a first-time flowering of a multi-year vegetative plant is not precisely defined but suggests a complex developmental trajectory. Environmental factors such as indoor versus outdoor cultivation can also lead to significant variations in expressed chemotypes, including differences in oxidized or degraded cannabinoids and terpene diversity, even in genetically identical plants.

**3. Overall Plant Vigor During and After Flowering:** Flowering is a highly resource-intensive process that signals the end of the life cycle for annual plants like cannabis in natural settings. An old mother plant, having been maintained vegetatively for an extended period, may exhibit a significant decline in overall vigor during the flowering process. It may struggle to sustain robust growth and could senesce rapidly post-harvest. The ability to successfully re-vegetate such a plant after it has been flowered would likely be severely compromised compared to a younger, more resilient plant.

## VI. Advanced Strategies for Rejuvenation and Long-Term Genetic Preservation

For valuable cannabis genetics maintained as mother plants, long-term viability can be threatened by the cumulative effects of aging, pathogen accumulation, and potential genetic instability. Advanced horticultural and biotechnological strategies offer pathways for rejuvenating mother lines and ensuring their long-term preservation.

### A. Tissue Culture and Micropropagation for Rejuvenating Mother Lines

Tissue culture, or micropropagation, involves growing plant cells, tissues, or organs in a sterile laboratory environment on a nutrient medium. It is a powerful tool for rapidly producing large numbers of genetically uniform and often disease-free plantlets from a selected mother plant. This technique is invaluable for preserving elite cannabis genetics and scaling up production.

However, long-term cannabis micropropagation is not without challenges. **Culture decline**, characterized by a gradual loss of vigor, reduced multiplication rates, and increased physiological disorders like hyperhydricity (a condition where tissues become water-soaked and translucent ), can occur over repeated subcultures. This decline can be attributed to various factors, including the accumulation of stress in vitro, nutrient imbalances in the media, and genetic or epigenetic changes.

**Somaclonal variation**, which refers to genetic or epigenetic changes that arise during tissue culture, is another concern. Studies have shown that somatic mutations can accumulate in micropropagated cannabis with an increasing number of subcultures, and some of these mutations have been identified in genes related to cannabinoid and terpene synthesis, potentially affecting the chemotype of the propagated plants. Strategies to mitigate somaclonal variation include minimizing the number of subcultures from a single explant line and careful selection of the initial explant material.

The **source of the explant** from the mother plant significantly influences in vitro performance and rejuvenation potential. Research on mature (2.5-year-old) cannabis mother plants has demonstrated that nodal segments taken from the basal (lower) portions of the plant exhibit better multiplication rates and produce plantlets with more juvenile morphological characteristics (e.g., shorter, wider leaves, indicative of rejuvenation) compared to explants from apical (upper) regions. This suggests that basal tissues may retain a higher degree of juvenility or regenerative capacity, possibly due to differences in endogenous phytohormone levels, sugar content, or epigenetic status compared to the more differentiated or stressed apical tissues. Apical parts may also harbor a higher load of accumulated somatic mutations.

One of the most significant advantages of tissue culture, particularly **meristem culture** (using the apical dome, which is often pathogen-free), is its ability to eradicate systemic pathogens, including viruses and viroids like HLVd, from infected mother lines. This "cleaning" process can restore vigor and productivity to a genetic line that had declined due to pathogen load.

It is important to understand that while tissue culture can "rejuvenate" a mother line by restoring physiological vigor (e.g., through a more balanced hormonal state in new plantlets) and by eliminating pathogens, it does not reverse accumulated somatic mutations present in the source explant. Indeed, the process of micropropagation itself involves numerous cell divisions, which can be an opportunity for new mutations to arise. Thus, the "rejuvenation" achieved is primarily physiological and sanitary, rather than a complete genetic reset to the original seedling state. This underscores the critical importance of selecting the healthiest possible explant material, preferably from more juvenile tissues if aiming for rejuvenation, and understanding that tissue culture is a tool for propagation and cleaning, not for erasing genetic history.

### B. Somatic Embryogenesis as a Potential Rejuvenation Pathway

Somatic embryogenesis (SE) is an advanced in vitro technique where somatic (non-reproductive) cells are induced to differentiate into structures that resemble zygotic embryos, termed somatic embryos. These somatic embryos can then germinate and develop into complete plantlets. SE offers a pathway for mass multiplication of genetically uniform plant material and is considered a powerful tool in plant biotechnology.

The success of SE is highly dependent on factors such as the specific plant growth regulators (hormones) used in the culture medium, the genetic makeup of the donor plant, the type of explant tissue chosen, and the overall culture conditions. While SE protocols are well-established for many horticultural and forest tree species, its application to *Cannabis sativa* is still an area of active research and development. Cannabis is generally considered relatively recalcitrant to de novo regeneration pathways like SE. However, some studies have reported successful shoot organogenesis or somatic embryogenesis, often from leaf or hypocotyl explants, though regeneration frequencies can be variable.

Theoretically, SE could offer a more profound degree of rejuvenation compared to organogenesis from pre-existing meristems. The process involves a more complete dedifferentiation and redifferentiation of cells, which might lead to a more significant resetting of certain epigenetic marks associated with aging or long-term vegetative stress. However, a common step in many SE protocols is an intervening callus phase (an undifferentiated mass of cells), which itself can be prone to somaclonal variation, including genetic mutations and epigenetic instability. Therefore, while SE holds promise as a rejuvenation technique, careful optimization and genetic fidelity assessment would be crucial for its reliable application in cannabis.

### C. Cryopreservation for Long-Term Genetic Archiving

Cryopreservation, the storage of biological material at ultra-low temperatures (typically in liquid nitrogen at -196°C), offers the most robust method for the long-term preservation of plant genetic resources, including elite cannabis mother lines. At these temperatures, all metabolic activity and cell division are arrested, effectively halting the processes of aging and the accumulation of new somatic mutations.

Given that cannabis appears susceptible to the accumulation of somatic mutations during normal vegetative growth and through successive clonal propagations (both conventional and micropropagation) , cryopreservation of shoot tips or other suitable propagules from the original elite plant (ideally at a young physiological age) can serve as a secure genetic backup. This cryopreserved material can later be thawed and regenerated, often through tissue culture techniques, to re-establish the mother line with a genetic makeup closer to its original state than might be found after many years of continuous cultivation and cloning. Research has demonstrated the feasibility of cryopreserving and successfully regrowing cannabis shoot tips, making this a viable strategy for safeguarding valuable genetics against loss due to disease, accidental destruction, or the long-term effects of clonal aging.

## VII. Synthesis and Recommendations for Cultivators and Researchers

The long-term maintenance of cannabis mother plants presents a unique intersection of horticultural art and plant science. While clonal propagation offers the allure of genetic perpetuity, the individual mother plant remains a biological entity subject to the inexorable processes of aging. Understanding these processes and implementing informed management strategies are crucial for maximizing the productive lifespan of mother stock and ensuring the consistent quality of clonal offspring.

### A. Key Considerations for Extending Mother Plant Lifespan

Cultivators aiming to extend the productive lifespan of their mother plants must balance the continuous demand for cuttings with the physiological limitations of an aging plant. Proactive and meticulous management is key. This includes providing tailored nutrition that meets the demands of constant vegetative growth while avoiding excesses that can lead to imbalances or toxicity. Optimizing environmental conditions—light quality and quantity, temperature, humidity, and airflow—minimizes stress and supports robust health. Regular, strategic pruning not only provides cuttings but also shapes the plant for optimal light exposure and air circulation, and can help manage its size and encourage new, vigorous growth. Diligent root care, including periodic root pruning and repotting, is essential to prevent root-bound conditions and maintain efficient nutrient and water uptake. Crucially, a comprehensive pest and pathogen management program, including strict sanitation and regular inspections, is non-negotiable to prevent the establishment and spread of diseases that can cripple a mother plant.

Despite the best care, it must be acknowledged that age-related decline in vigor, rooting potential of cuttings, and overall productivity is, to some extent, inevitable. Therefore, a well-defined mother stock rotation plan is a fundamental component of any sustainable cannabis cultivation operation. This typically involves replacing older mother plants with fresh, vigorous stock (often clones from elite, younger mothers or tissue-cultured plantlets) on a regular schedule, commonly ranging from every 3-6 months to annually, depending on the intensity of use and observed performance. Vigilant monitoring for early signs of decline—such as reduced cutting vigor, slower rooting times, increased susceptibility to pests or diseases, or undesirable changes in growth morphology—is critical for making timely decisions about retirement and replacement.

### B. Integrated Strategies for Managing Aged Mother Stock

For particularly valuable or unique genetic lines where extending the use of an aging mother plant is desired, or when transitioning such plants, an integrated approach is recommended:

* **Tiered Mother Stock System:** Implementing a hierarchical system where elite "grandparent" stock (often derived from tissue culture and carefully maintained with minimal cutting pressure) is used to generate "parent" mother plants for bulk clone production can significantly reduce the effective "age" and accumulated stress on the plants providing the majority of cuttings.
* **Strategic Re-vegetation:** If genetics are highly valuable and new mother stock is not yet available, re-vegetation can be considered to extend the utility of a healthy, moderately aged plant for an additional cycle or two. However, this should not be viewed as a long-term solution for very old or declining plants, given the stress involved and potential for diminishing returns.
* **Selective Flowering of Aged Mothers:** Inducing flowering in an aged mother plant is generally best reserved for situations where the genetic line is being retired and a final assessment of its floral characteristics is desired, or for specific breeding purposes. Cultivators should be prepared for potentially suboptimal outcomes in terms of yield or quality compared to plants flowered in their prime.
* **Periodic Tissue Culture Rejuvenation:** For high-value cultivars, periodically cycling the genetic line through tissue culture, particularly meristem culture, can serve to eliminate accumulated pathogens and potentially "reset" some aspects of physiological aging by propagating from more juvenile explant sources (e.g., basal nodes). This can help maintain the health and vigor of the genetic line over many years.

### C. Future Research Directions in Cannabis Aging and Rejuvenation

Despite the growing sophistication of cannabis cultivation, several areas concerning mother plant aging and rejuvenation warrant further scientific investigation:

* **Biomarkers of Vegetative Senescence:** There is a need for the identification and validation of reliable, easily measurable physiological and molecular biomarkers that can accurately indicate the onset and progression of vegetative senescence in cannabis mother plants. This would allow for more objective assessments of mother plant health and better-timed replacement.
* **Longitudinal Hormonal Profiling:** Detailed studies tracking the endogenous levels and ratios of key phytohormones (auxins, cytokinins, ABA, ethylene, gibberellins) in cannabis mother plants over extended periods (years) of vegetative growth are needed. Correlating these hormonal profiles with cutting performance (rooting success, vigor) and overall plant health would provide critical insights.
* **Genetic and Epigenetic Stability in Situ:** While micropropagation studies provide valuable data on mutation accumulation , research is needed to quantify somatic mutation rates and epigenetic drift directly in long-term, conventionally maintained mother plants and to determine their precise impact on phenotypic traits like yield and chemotype.
* **Optimizing Re-vegetation for Aged Plants:** Research into techniques, possibly including targeted hormonal applications or specific environmental conditioning, to improve the success rate and vigor of re-vegetated older cannabis plants could be beneficial for preserving rare genetics.
* **Flowering Outcomes from Aged Mothers:** Controlled, comparative studies are needed to systematically evaluate flower yield, detailed cannabinoid and terpene profiles, and overall quality from mother plants of significantly different ages (e.g., 1 year vs. 3 years vs. 5+ years) that have been kept continuously vegetative before flowering. This would provide data beyond anecdotal reports.
* **Somatic Embryogenesis in Cannabis:** Continued research to develop and refine efficient and reliable somatic embryogenesis protocols for diverse cannabis genotypes could offer a powerful tool for true rejuvenation and mass propagation, provided somaclonal variation can be managed.
* **Telomere Dynamics:** Investigations into telomere length and telomerase activity in relation to clonal aging and long-term vegetative maintenance in cannabis are currently lacking and could shed light on fundamental aging mechanisms.
* **Transcriptomic and Metabolomic Analyses:** Comprehensive transcriptomic and metabolomic studies focusing specifically on the aging process in continuously vegetative cannabis mother plants would help elucidate the molecular pathways involved in their decline and identify potential targets for intervention.

By addressing these research gaps, the cannabis industry and scientific community can develop more effective strategies for maintaining the health, productivity, and genetic integrity of valuable mother plant lines, ensuring a sustainable supply of high-quality cannabis for medicinal and recreational use.

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