GROWING GUIDE: THE FIRST SEVEN DAYS

PARAMETERS FOR INDOOR CULTIVATION OF GENERATION ZERO TISSUE-CULTURED CANNABIS PLANTLETS

CONCEPTION NURSERIES

EXECUTIVE SUMMARY

Acclimation losses come from plant stress. Too low humidity, too much light, too much fertilizer, and too high a VPD will lead to acclimation losses with tissue culture plantlets.

IDEAL LEVELS FOR ACCLIMATION SUCCESS

Humidity: 75 - 82%

Light Intensity: 170 - 250 micromoles

Fertilizer Electrical Conductivity (EC): 1.2 - 1.9 EC

Vapor pressure deficit (VPD): 0.60 - 0.89 kPa

HIGH** Humidity, **LOW** Light, **LOW** Fertilizer, and **LOW** Vapor Pressure Deficit **(HLLL)

INTRODUCTION

Problem

Traditional clonal nurseries are plagued by problems such as pest pressure, diseases, and clonal decay. In the face of ongoing price compression in the legal cannabis market, growers find little room for error, making disease-free production and the preservation of unique cannabis genetics critical for maintaining profitability. Commercial cannabis cultivators are adopting the mainstream technology of tissue culture to help mitigate the limitations of traditional cannabis cloning.

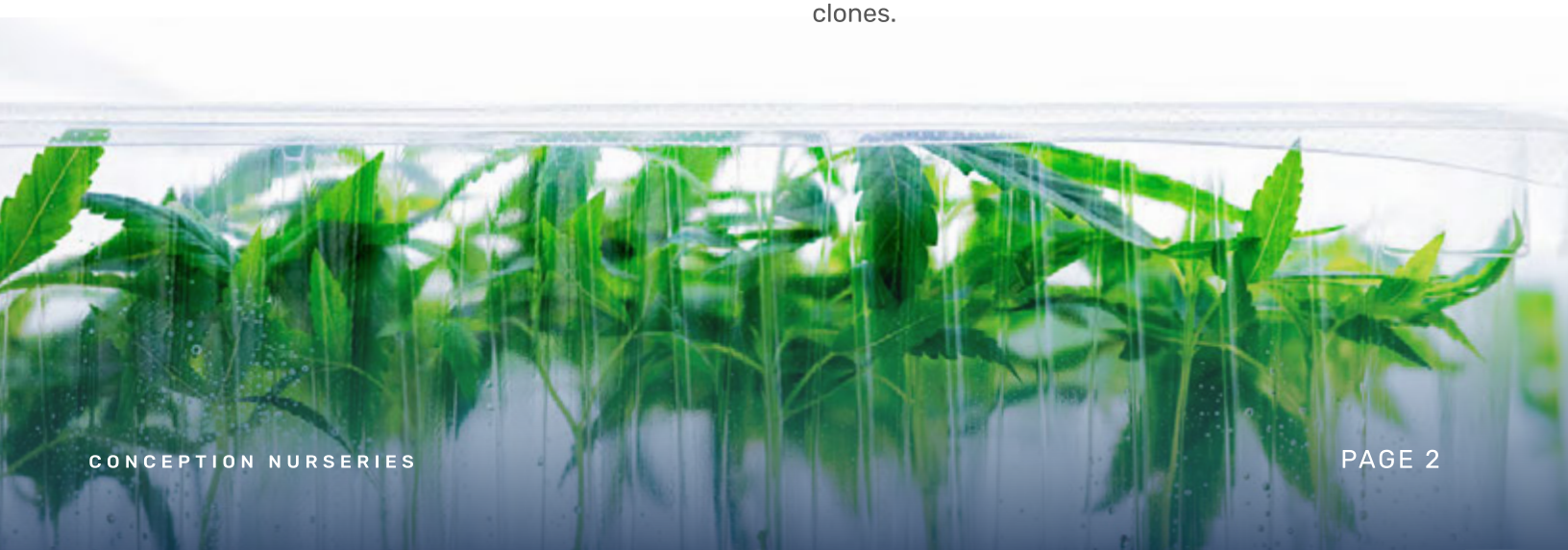
Generation-zero plantlets produced via tissue culture (TC) significantly outperform traditional cuttings by offering enhanced vigor, consistent structure, and a pest-free start. These qualities reduce labor and remove the need for growth manipulation via topping and low-stress training. This technology curtails labor costs and pathogen risks incurred by conventional cloning and fosters optimal plant performance through a robust, disease-free growth environment.

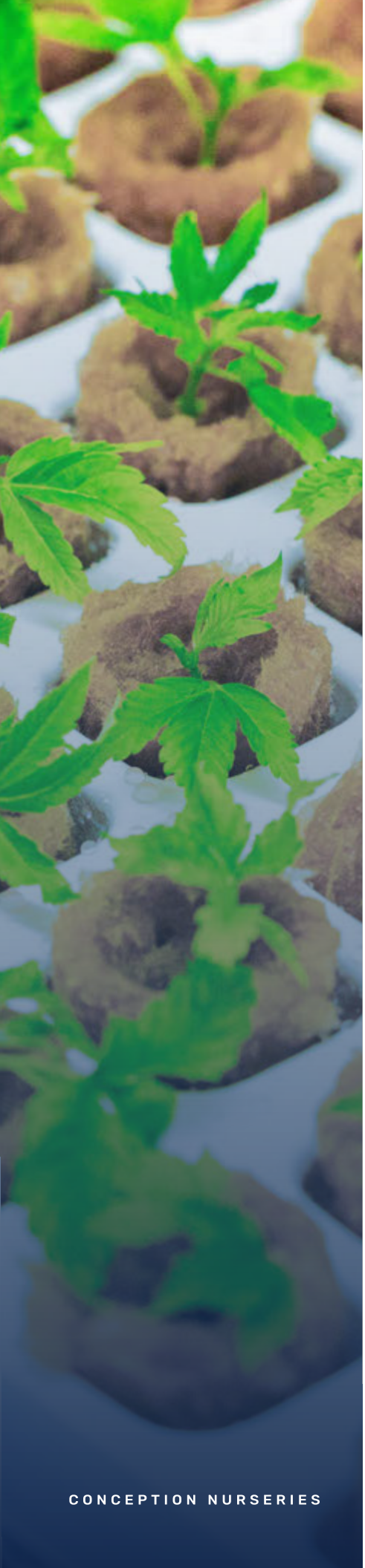
Indoor cultivation sites are primarily optimized for flower production and the acclimation of traditional clones, often lacking the distinct environmental needs required for TC plantlet acclimation. This often leads to unnecessary plant loss when introducing tissue culture clones to a traditional cannabis cultivation environment.

Solution

The first few days of acclimatization are crucial for tissue culture clones, which are particularly vulnerable to abiotic stress during this period of time. Ensuring a successful transition requires strategically reducing this stress by carefully modulating the acclimatization environment to include high humidity, low light intensity, the application of fertigation at low concentration levels, and the meticulous regulation of vapor pressure deficit (VDP) to optimal low levels. This precise control of environmental parameters is not merely beneficial, but essential, as a gentle acclimation to the cultivation process is the foundation for tissue culture clones to exhibit their full potential.

Cultivators can mitigate the risk of plant loss by investing in a methodical and gradual acclimatization process for tissue culture clones. Gently and properly acclimated tissue culture clones display remarkable resilience and thrive in traditional cultivation environments, fully maximizing their performance. In contrast, clones rushed through the acclimatization process often encounter difficulties adjusting to cultivation conditions, leading to suboptimal growth and a failure to realize their full potential. This stark difference shows that a well-planned and executed acclimatization strategy is the cornerstone for achieving success in cultivating tissue culture clones.





ACCLIMATING HARDENED PLANTS TO INDOOR GROWING CONDITIONS

The acclimatization process aims to **minimize abiotic and biotic stress on the clones** during this one-week transition phase and de-risk the possibility of unnecessary plant loss.

Tissue culture clones are propagated in a highly controlled environment with narrow fluctuation ranges for environmental parameters. Lab plantlets have physiological and anatomical traits that require gradual acclimatization to the field, greenhouse, or indoors. These changes are in **stomata regulation, density and form, cuticle development, epicuticular waxes, thickening of the leaves, and chlorophyll content**.

COMMERCIAL PRODUCTION ENVIRONMENTS

- Low Humidity
- High Light Levels
- Higher Fertilizer Rates
- High Vapor Pressure Deficit (VPD)

While these environmental and chemical conditions drive outstanding production and flower quality, these conditions can also lead to extremely low tissue culture clone acclimation success rates.

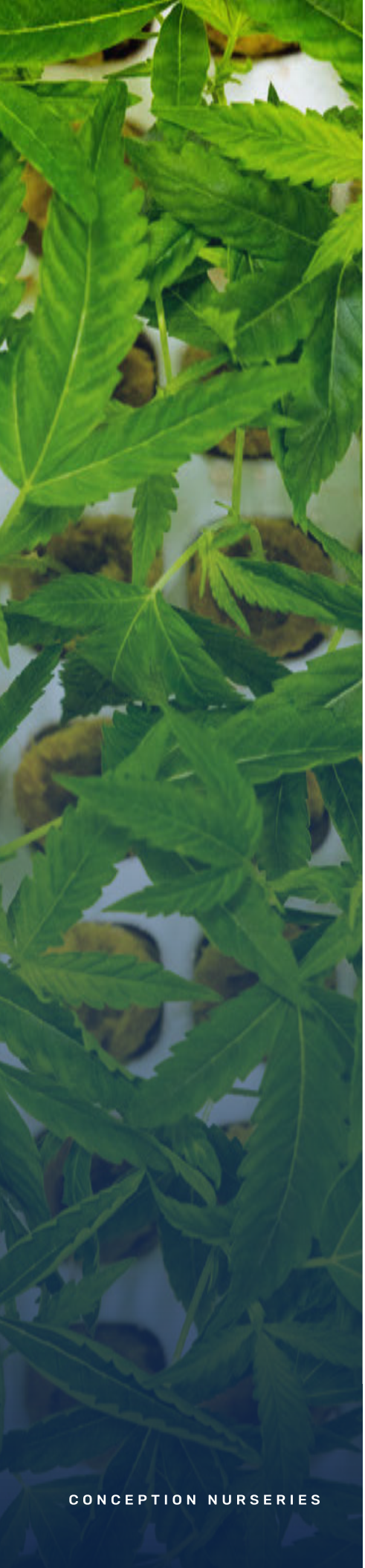
To prevent these low acclimatization rates, Conception Nurseries created a plan for strategically adjusting these environmental and chemical conditions to develop a foundation for the successful acclimation of tissue culture clones. Here, we present the key factors of the acclimatization process that have the most significant effect on plant survival and success:

HUMIDITY CONTROL

FERTILIZER STRENGTH

LIGHTING LEVELS

VAPOR PRESSURE DEFICIT (VPD) LEVELS



ACCLIMATING HARDENED PLANTS TO INDOOR GROWING CONDITIONS (CONTINUED)

CONCEPTION NURSERIES

- High Humidity (over 70%)
- Low Light Levels (PPFD under 300 micromoles/m2)
- Low Fertilizer Rates (under 2.0 EC)
- Low Vapor Pressure Deficit (VPD) (under 0.9 kPa)

***HLLL** (high, low, low, low)*

A gentle start to the acclimatization process is an investment in the long-term growth and performance of the tissue culture clones. By gently introducing tissue culture clones to the traditional cultivation setting, successful cultivators can minimize unnecessary plant loss and de-risk the process of slowly ramping up abiotic stress on the plant to produce more vigorous growth and performance.

The first few days of the acclimatization process are the most volatile, and utilizing this gentle start allows a more efficient hardening-off process by minimizing abiotic stress on the clones. After about 6-8 days, it is expected to observe increased turgidity and the development of new growth. This will indicate that the clones have begun adapting to their new environment and are prepared for slightly elevated levels of abiotic stress and to endure slightly higher light intensity and slightly higher fertigation strength. The clones at this point are also more tolerant to fluctuations in humidity and VPD.

HUMIDITY

A high-humidity environment needs to be created to begin the acclimatization process. Slow tapering from very high to moderate humidity is a prerequisite for successfully establishing micro-propagated plants. If a devoted nursery space with equipment in place to create high humidity levels is not always available, deploying misting systems and traditional clone domes can be used to develop high-humidity environments without allocating extra nursery space or funds.

The optimal relative humidity (RH) should be **75% or at least 70%.** If the humidity falls below 70%, plant loss will occur. Electronic hygrometers are an option to measure humidity and ambient temperature to estimate the RH. This gradual decrease in humidity should happen over the span of about 5-10 days.

LIGHTING

Tissue culture clones cannot be immediately exposed to the natural lighting of an outdoor cultivation environment or the high-power LED lighting of an indoor cultivation environment. The in vitro plantlets only have a few palisade cells (located on the outermost layer of the leaves) that are too small to receive light effectively.

As such, it is necessary to provide the tissue culture clones with a **mild light intensity (around 170-250 PPFD)** during the beginning of the acclimatization phase.

One way to accomplish these light intensities is deploying shade cloths and peripheral lighting systems, like clone bars or simple LED strips, to dim the light source or create a new source of low-intensity lighting at the beginning of the acclimatization process.

There are a few specialized tools for measuring PPFD. Quantum sensors are small devices that measure PAR using light detectors. A PAR meter is a device that measures PAR over a specific area. They are more accurate but more expensive than quantum sensors. Light meter measures the intensity of light but is less precise since it measures all kinds of light, not just PAR.

Until the second week, the light PPFD must be under 300 micromoles. After the first week, lighting intensity should be adjusted to a cultivator's preferred range for the vegetative cycle at a gentle pace in parallel with the physiological acclimation and development of the TC clones.

FERTILIZER STRENGTH

Rooting plantlets that have just been transferred to in vivo are weak and unable to absorb nutrients optimally, which makes tissue culture clones very sensitive to shock from fertigate solutions with a high charge.

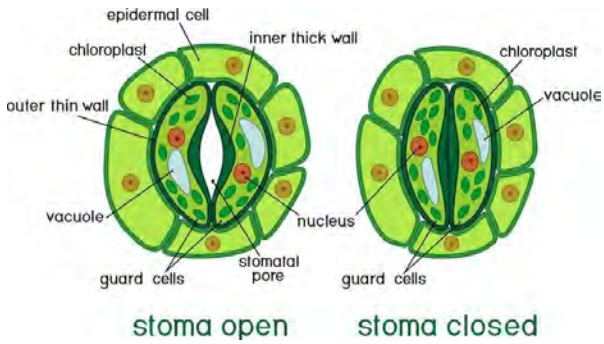
An **EC of 1.2-1.9 is the ideal range for fertigation strength** during the acclimatization process, dependent on the charge of the transplanting substrate.

If planting into an inert substrate like Rockwool, it is best to be close to the higher end of the range. If planting into a pre-charged substrate, stay on the lower end of the range. As the clones acclimatize in their new up-potting vessel and develop more robust root systems, they can tolerate higher concentrations of fertilizer solution.

CN clone plants thrive at an EC of under 2.0. High EC will keep the plant from being able to intake water. If the EC is too high, decrease the frequency and/or concentration of fertilization. If the levels are far too high, use leaching for the fastest results (using water to dissolve the salts in your substrate). Fertilizer levels can be measured with a soil EC meter. Increasing fertilizer strength to a cultivator's preferred range for the vegetative cycle should occur gently in parallel with the physiological acclimation and development of the TC clones.

VAPOR PRESSURE DEFICIT LEVELS (VPD)

Cannabis plants inhale Co2 and exhale oxygen and moisture through openings in the bottom of the leaves called stomata. Optimizing VPD allows the plants to open their stomata to the ideal level for transpiration and photosynthesis based on their plant stage.



VPD is the variable used to measure the difference between the amount of moisture currently in the atmosphere versus the total amount of moisture the atmosphere can hold. VPD drives the process of water movement through the plant and is a crucial variable to consider when discussing the transpiration rate of TC clones. TC clones have limited stomatal function and underdeveloped cuticles and epicuticle waxes, increasing the desiccation caused by high VPD.

In addition, lower VPD levels also pose a concern for the acclimation process of TC clones, as lower VPD levels keep the clones from adequately respiring, slowing down the acclimation process. Too high or low a VPD can lead to plants stopping Co2 intake, taking in too much water or nutrients, inadequate transpiration, increased susceptibility to fungal and pest pressure, and low TC acclimation success rate.

Managing VPD is essential to avoid exposing TC clones to additional abiotic stress during the acclimation period and to maintain an efficient pace in the hardening-off aspect of acclimation. The air in the growing environment should be near saturation (VPD between 0.6 and 0.9 kPa.) It should not go above 1.0 kPa. Measuring VPD does not require a specific tool. Instead, gauge the temperature of the air and the relative humidity to find what the VPD value is.

Use the %RH chart below as a reference. If your VPD is outside its **optimal range**, adjust your grow room's temperature and/or humidity.

°C	RELATIVE HUMIDITY %RH					°F
	70%	75%	80%	85%	90%	
22°	0.79	0.66	0.53	0.40	0.26	71.6°
23°	0.84	0.70	0.56	0.42	0.28	73.4°
24°	0.90	0.75	0.60	0.45	0.30	75.2°
25°	0.95	0.79	0.63	0.48	0.32	77.0°
26°	1.01	0.84	0.67	0.50	0.34	78.8°
27°	1.07	0.89	0.71	0.54	0.36	80.6°
28°	1.13	0.95	0.76	0.57	0.38	82.4°
29°	1.20	1	0.80	0.60	0.40	84.2°

WATERING

Overwatering is also one of the more significant issues in acclimatization, but it is more difficult to measure since tensiometers can cause root damage in small plugs. Carefully monitor the watering cadence to avoid overwatering.

CONCLUSION

Acclimatizing tissue culture clones is a multi-step process, where each step allows for specific developmental changes in the clones as they acclimate to their new environment. Environmental parameters are the driving forces guiding tissue culture clones through the process. Appropriate manipulation of these parameters is necessary for successful acclimatization and creating vigorous plant growth as efficiently as possible.

We emphasize the first few days of acclimatization when tissue culture clones are most sensitive, and minimizing abiotic stress is critical to ensure successful acclimatization and plant performance. Successful cultivators minimize abiotic stresses by beginning the acclimatization process with **high humidity levels, low levels of light intensity, feeding clones fertigate with low strength, and maintaining low levels of vapor pressure deficit (HLLL).** Investing in a gentle, slow start to acclimatizing tissue culture clones is the foundation for success in the later stages of the cultivation process.

HLLL

For optimum success, use the acronym HLLL (high, low, low, low) to guide the process during the first 7 days.

INDOOR CULTIVATION	PARAMETER	MINIMUM	IDEAL
High	**Humidity**	Above 70%	75-82%
Low	**Light Intensity**	Below 300	170-250 micromoles
Low	**EC Strength**	Below 2.0 EC	1.2-1.9 EC
Low	**VPD**	Below 0.9 kPa	0.60 – 0.89 kPa

*Target the conditions in the ideal range. Anticipate significant plant loss if straying from the minimums during week 1

GLOSSARY

EC (Electrical Conductivity)

The metric for measuring fertilizer is EC (electrical conductivity). EC is the level of dissolved salts in a solution. EC measures the total salts in your plant, not just the fertilizer you've added.

PPFD (Photosynthetic Photon Flux Density)

PPFD measures the amount of PAR, the wavelengths affecting photosynthesis, that actually make it to the plant. This is important to measure because the PPFD directly below your lights will be significantly higher than what it'll be at the level of your seedlings, so you can't rely on what your lights are rated.

To measure light, we use PPFD (photosynthetic photon flux density). It's a more specialized form of measurement for plants; traditional means of measuring light, like LUX, only measure how humans perceive light.

RH (Relative Humidity)

Humidity is measured in RH (relative humidity). RH is a ratio written as a percent of how much moisture is in the air compared to what it would be if the air were completely saturated. It's measured this way because the saturation point changes depending on the temperature.

VPD (Vapor Pressure Deficit)

VPD (vapor pressure deficit) measures how much more or less water the air in your grow room can hold and how much the air will dry your plants. So, a high VPD means the air could hold more water, and a low VPD means the air is almost saturated.

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