Strains
Selection Plan

Selecting Cannabis Strain Varieties

Planning strain varieties to produce is essential to meet demands for, produce quality cannabis, and to ensure consistency of quality. Feedback from retail distributors and the cultivation team shall be combined with comprehensive test results to set production goals for the company. In addition, a given strain's ease of propagation and overall yield must be taken into account in order to reach production goals and maximize energy and space efficiency within the company. The cultivation manager shall plan a cultivation calendar in order to meet demand and production goals. The following represent other important strain differentiations that will be taken into account by the cultivation manager:

Cannabinoids

There are over 480 natural components found within the cannabis plant, of which at least 85 have been determined to be cannabinoids. Cannabinoids are chemicals found in animals and other plants as well, but nowhere are they more abundant and effective than in the cannabis plant. The most well-known and researched of these is THC or delta-9-tetrahydrocannabinol. THC is the substance primarily responsible for the psychoactive effects of cannabis.

Like opiates and opiate derived pharmaceuticals, cannabinoids affect the user by binding with receptors within the cells of the body and different parts of the central nervous system. There are at least two kinds of cannabinoid receptors found to date, termed CB1 and CB2. Anandamide is a cannabinoid-like substance found within the brain commonly referred to as the “Bliss Molecule.” Naturally occurring anadamide binds to CB1 receptors. Other naturally occurring substances that bind to CB1 have recently been discovered, and these, together with the receptors, have been termed the endogenous cannabinoid system or endocannabinoid system.

The effects of THC are heavily influenced by the other components of the plant, most particularly, other cannabinoids. Differences between the cannabinoids found within the cannabis plant are determined by the extent to which they are psychologically active and the other medicinal benefits they correspond with. For example, CBG, CBC and CBD are not known to have a psychological effect, however they have been proven to have multiple medicinal benefits, while THC and CBN are cannabinoids often associated with the psychological effect of cannabis. Most cannabinoids are multifaceted with a wide array of effects and benefits.

The company cultivation manager shall review test results for every batch in order to ensure that multiple strains are produced with varying cannabinoid profiles in order to properly and accurately provide medicines to treat multiple consumers with different needs. By combining different cannabinoids and different terpene profiles, the possibilities for medicinal healing are vast. The company cultivation manager shall utilize cannabinoid profiles when planning for strain production at all times. The following cannabinoids are all known to provide different effects and medicinal benefits:

- **THC**

  Delta 9 Tetrahydrocannabinol is the cannabinoid that gives cannabis the majority of its psychoactive affects. Cannabis has been bred for high levels of THC, and we are only now starting to breed for complimentary cannabinoids. THC can be used to treat pain, nausea, tumors, and ADHD.

- **THC-A**

  THC-A is the most common cannabinoid found in cannabis. It is not psychoactive and has an array of medicinal effects including anti-tumor, anti-insomnia, anti-inflammatory, and anti-spasmodic.

- **CBN**

  (Cannabinol) As THC oxidizes from exposure to heat and light, it turns into CBN. CBN is only mildly psychoactive and highly sedative.

- **CGB**

  Cannabigerol is a non-psychoactive cannabinoid that stimulates brain cell development and bone growth. It is also antibacterial, anti-insomnia, and anti-tumor.

. **CBC**

  Cannabichromene has been shown to be 10 times more effective than CBD for treating anxiety. CBC also stimulates bone growth. It is non-psychoactive.

. **CBD**

  Highly effective in treating epilepsy and MS, cannabidiol is non-psychoactive. CBD is as effective at treating tumors and pain as THC, however very useful for children and to others whom do not wish for the psychoactive effects of THC. CBD can treat diabetes by lowering blood sugar and is very effective in treating stress and insomnia.

. **CBD-A**

  CBD-A is more commonly found in the ruderalis varieties, which are often bred with sativa and indica varieties for their auto flowering abilities. CBDA has been linked to anti-tumor and anti-inflammatory effects.

Varieties of Cannabis

. Cannabis Sativa

Cannabis sativa is the tallest variety of cannabis. All other varieties most likely evolved from the sativa plant. Most hemp is actually sativa. Sativa varieties are thin and wispy, most likely from evolving in hotter regions of the world where adequate airflow between branches was necessary to remain disease free. 100% sativa strains take up to six months to flower and can grow extremely tall making true sativa unsuitable for indoor and commercial production. The sativa strains commonly grown are actually hybrids that have been bred with indica varieties to shorten both their height and flowering time. Sativa produces a cerebral effect that can be energizing, followed by an increase in appetite. Sativa hybrids are very helpful for anyone experiencing loss of appetite such as those undergoing chemotherapy or patients with HIV/AIDS.

. Cannabis Indica

Cannabis indica is short and bushy with thick stems. Most likely evolving in the cooler regions of Asia and Afghanistan, cannabis indica has a short flowering time, most likely to complete its reproductive cycle prior to freezing conditions. Most varieties of cannabis are a derived form of indica, and its cannabinoid profiles are well-balanced producing significant levels of THC, CBD,

and CBN. Indicas produce a body-centered effect that allows relaxation, quality rest, and pain relief for consumers.

. Cannabis Ruderalis

Cannabis reuderalis is of very poor quality and is only grown in hybrid form with sativa or indica due to its auto flowering capabilities. For the most part, ruderalis hybrids should be avoided in indoor cultivation. Ruderalis is the shortest cannabis variety, and it has minimal branching. Avoid all seeds and strains that state they are auto flowering.

. CBD Varieties

CBD varieties are now being bred as they offer many of the medicinal benefits of cannabis with little to no psychological effects making them suitable for children and adults who wish to remain clear headed. CBD effects on THC can be noticed with as little as 1% CBD, however, the ratio of THC to CBD can be widely influenced and tailored to create the desired effect for a taste. One-to-one ratio strains often have the best of both worlds where the psycho effects of THC are diminished by CBDs, however, all the medicinal effects from THC remain intact.

Breeding Guide

Genetic model of chemotype - Overview

Genetic studies demonstrate control for cannabinoid accumulation in published, peer reviewed literature. Principal research was completed and published over four papers by de Meijer et al. 2003-2009.

Key findings:

- Accumulation of CBG, CDB, CBC and THC is under genetic control
- Genetic variation, needed for classic breeding to succeed, has been demonstrated to exist.
- Studies demonstrate clear and repeatable segregation for accumulation of cannabinoids, emphasizes need for genetic diversity in genetic library/collection
- Demonstrated that breeding efforts can be utilized to stabilize chemotype
- Published genetic model below:

. Genetic model of high CBC lines

CBC is not an accumulated cannabinoid like THC or CBD. CBC is not accumulated in floral tissue, it is found in leaves. CBC is a juvenility linked compound that the plant loses the ability to produce as it moves toward sexual maturity. Rarely found are plants that do not lose ability to create CBC, especially in the first 60 days of plant growth. Example I figured (d) maintains CBC vs. normal (b) loses CBC.

The rare plants can be found through repeated chemotype analysis; requires close collaboration with analytical lab to regularly test young plants.

CBC retention is under genetic control and can be stabilized in self-progeny. Literature does not elucidate genetic control in crosses or other genetic situations (though likely recessive). Discovered in Afghan and Korean lines (de Meijer, 2009)

Challenges:
Low amounts found: 0-4% w/w (15-100% CBC by weight of total cannabinoids) %CBC increases with decreased light. To maximize yield, research needs to balance plant growth and CBC accumulation.

. Genetic model of high THC/CBD lines

One genetic pathway controls ratios of THC/CBD. Leads to clear differentiation within a family as shown (figure right). Published report that THC/CBD ratios are stable in the plant in the first true leaves until maturity. This allows the chemotyping of seedlings and discarding of unwanted proportion of family when plants are small; efficient breeding.
Key to breeding progress for % accumulation of THC/CBD is intermating of non-drug and drug strains.

Requires repeated chemotype analysis with first true leaves and then confirmation of % accumulation of mature floral structure.
Pure CBD lines may be difficult to achieve due to shared genetic pathway with THC. Highest CBD lines may accumulate a minor percentage of CBC, CBG, and/or THC. Similarly, high THC lines may accumulate a minor percentage of CBD, CBC, and/or CBG. Challenge is to improve accumulation and purity with breeding.

- **Tissue Culture support of breeding/production plan**

  Methods described in great detail in Lata et al., 2009; specific method optimized for Cannabis. Proven genetic and chemotype stability over several generations. Protocols/Methods demonstrated commonly online; sterile technique required utilizing clean air hood.

  Tissue Culture procedures can be utilized for:

  - Multiplication/production aid for clonal propagation
  - Maintain key clean lines/genetics in living library/backup; creating a living germplasm bank
  - Seed germination aid – can be utilized for embryo extraction from older/degraded seed

Breeding 101

There are three main steps to breeding:

- **Evaluate**

  Chemotype - using analytical laboratory capabilities to quantify range of population. Identify all cannabinoids and secondary metabolites of interest. Phenotype (physical type) – quantify ranges in traits key to success of production and/or market: rate of growth, days to flower, leaf shape, leaf size, other aesthetics (purples, golds, etc.) or traits of interest or differentiation.

- **Select**

  Identify elite plants or high performers from population based on improved chemotype and phenotype.

- **Recombine**

  Mate only high performers based on improved chemotype and phenotype. Sow seeds and **Evaluate** progeny to begin the breeding cycle again.

- **Result**

  Advancing each generation beyond the previous generation in a continuous process.

Method for Stock Seed Establishment

- **Starting from purchased seed of landraces, varieties, or clones**
    - Sow seeds, recover 1-10 seedlings
    - Grow seedling, record important plant characteristics
        - Ex. leaf shape, size, growth rate, branching, days to flower, chemical phenotype, flower yield, chemical yield, etc.
    - Keep up to 6-10 desirable plants
    - Allow plants to mass/group pollinate within landrace/variety
    - Collect seed, record seed volume, germination test and store
    - Split seed for future utility
        - Internal genetic library, gemplasm bank – store seed cool and dry for later use
        - Utilize to create proprietary, feminized seed line

- **Starting from purchased clones**
    - Grow plants of known clones, record plant characteristics
        - Ex. leaf shape, size, growth rate, branching, days to flower, chemical phenotype, flower yield, chemical yield, etc.
    - Keep up to 4 desirable plants
    - Allow plants to mass/group pollinate
    - Collect seed, record seed volume, germination test and store
    - Split seed for future utility
        - Internal genetic library, gemplasm bank – store seed cool and dry for later use
        - Utilize to create proprietary, feminized seed line

Method for Establishing a Proprietary Feminized Seed Lines

- Sow 100 seeds from Stock Seed of each line.
- Complete chemotype analysis (at 1-3 true leaf stage)
    - Discard undesirable 25-75% of plants (percentage discarded will be goal and experience driven)
- Grow plants to flowering
    - Discard males when identified unless needed in breeding
    - Discard weak plants based on poor growth, non-flowering, etc.
- Evaluate female plants for flowering, chemical yield
    - Take cuttings to establish new proprietary clone/vegetative line (below) **OR**
- Select 3-5 individuals to intermate

    - Intermating of sister plants begins creation of feminized, genetically pure seed line

Repeat sowing 100 seeds, evaluation, and selection until plants closely resemble each other in desired chemotype and phenotype.

Method for Establishing a Proprietary Clone

- Take 10-20 cuttings from original, elite, selected plant to establish new/improved clone.
- Root $^1/_2$ of cuttings per convention and grow in production setting
    - Confirm evaluation of chemotype and chemical yield
    - Evaluate for growth rate, flowering characteristics, other key factors to production
- Initiate $^1/_2$ of cuttings from original selected plant into tissue culture for future utility (method above)
    - Keep tissue culture if line performs well in production, discard tissue culture if line fails.
- Begin process to convert line to proprietary, feminized seed form (above)