Science Societies

A first look at genetic mosaicism in cannabis

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Cannabis plants in the flower room at the BrantMed facility are kept under specific light and photoperiod conditions to initiate flowering. Photo by Kristian Adamek.

From afar, a good mosaic is as smooth and cohesive as a painting—it's only when you come closer that you can see the distinct tiles forming the bigger picture.

In plants, mosaicism refers to spontaneous somatic mutation in new growth that becomes fixed as a cell line progresses. That is, the apical tips of a tree branch, where new growth rapidly occurs, could accumulate one mutation, and then through division, pass that mutation on to newly generated cells. The most commonly studied example are long-lived perennial species like trees.

But new research in *The Plant Genome* (TPG) indicates that it's not just perennials that can accumulate somatic mutations. In fact, researchers at the University of Guelph and Université Laval discovered genetic mosaicism in a single 1.5-year-old *Cannabis sativa* mother plant (**https://doi.org/10.1002/tpg2.20169**).

Though preliminary, the discovery of such rapid mutations could have implications for cannabis producers, who rely on consistent quality from clonal propagations taken from valuable cannabis mother plants.

Cannabis in Canada

On 17 Oct. 2018, **Canada legalized cannabis** for recreational use for all adults over the age of 18. Federally licensed commercial cannabis production facilities sprang up virtually overnight. Most of those facilities base their production on one very important asset: the mother plant.

Co-authors Kristian Adamek and Davoud Torkamaneh take samples from the 'Honey Banana' plant at the BrantMed facility—the mother plant the research team used for samples and generating clones. Photo by Kristian Adamek.

A mother plant is the source for all the cannabis plantlets that an organization will produce since cannabis plants aren't derived from true seed. For one thing, producers of medicinal and recreational cannabis only want female plants. Females produce flowers, which produce trichomes, which produce the two most valuable cannabinoids, delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD).

For another, growing cannabis plantlets from seed is incredibly time and labor intensive. Instead, recreational and medicinal commercial cannabis producers keep mother plants in a vegetative state for two or more years using carefully monitored photoperiods and climate control conditions in the greenhouse. To grow a new plant, they typically cut a small branch from the top of a mother plant. That plantlet is potted up, put out in the greenhouse or indoor growing facility, producing valuable flowers and leaves.

Producers operate on the assumption that all the cuttings they take from the mother plant are genetic clones. But Davoud Torkamaneh, a professor at Université Laval with a public cannabis-breeding program, kept hearing from producers that cuttings taken from old mother plants just aren't as vigorous or productive as cuttings from their younger counterparts.

So Torkamaneh and his team investigated. Their industry partner BrantMed Inc. supplied them with mother plant from the Honey Banana cultivar, known for its high levels of THC. They took cuttings from the top, middle, and bottom of the one-and-a-half-year-old mother plant's canopy to compare their genetics.

The team extracted DNA from each of the three samples. They sequenced and then compared them to the public cannabis reference genome.

"We did very high-depth sequencing, around 50×. We took 50 copies of the same region just to make sure there's no sequencing errors, no genotyping errors," Torkamaneh explains. "When we started sequencing, there were millions of variants—that's just crazy!"

They even ran the sequencing twice with all new tissue samples to verify their results.

They found that samples from the top of the mother plant contained 592,000 unique variants compared with the reference genome. Tissue from the bottom of the plant contained 147,000 variants while the middle had 77,000 unique variants. Surprisingly, the middle section of the plant was the least different from the reference genome.

"Within one plant, we're seeing 1 to 2 million different variations," Torkamaneh says. "It's like looking at three plants all in one place."

Implications for Cannabis Production

Though a million variations sound formidable, the team's findings are preliminary. Researchers have studied somatic mutations most often in the context of long-lived perennials, particularly trees. In fact, another study in TPG outlines mosaicism in a 36-year-old clementine tree (**https://doi.org/10.1002/tpg2.20162**). The prevailing theory is that random somatic mutations provide these long-living species a means of adapting to their environment through mutation at the species level instead of the population level. That is, these perennial plants allow somatic mutations to accumulate, creating genetic variation within a single lineage of cells that can create better-suited offspring to the environment. This is the explanation a team of scientists offered in their studies of somatic mutations in Sitka spruce trees (**https://doi.org/10.1002/evl3.121**).

But conifers and clementines aren't typically used to create commercial medicinal and recreational compounds.

"In the case of cannabis, you're taking an annual plant and keeping it vegetative for an extended time, and creating what we hoped were genetically identical cuttings," Torkamaneh says. "People often consume the whole plant, and there's hundreds of different biochemical products. If the plant is not genetically stable, then it could be that the product is not always uniform."

Torkamaneh's team tested whether these mutations are in "high impact" areas of the cannabis genome associated with THC and terpene pathways. Cannabis produces more than 100 different smelly terpene compounds, creating different aromas based on their relative ratios in the plant. The team found that about 13% of mutations were present in coding regions of the cannabis genome.

“On the one hand, if these mutations make the plant stop producing compounds such as cannabinoids or terpenes, that’s a problem,” says Isabel Desgagné-Penix, Canada Research Chair on Plant Specialized Metabolism and professor of biochemistry at the University of Québec at Trois-Rivières. “But if these mutations can produce more cannabinoids, or new cannabinoids, they could be really valuable for producers.”

Of course, it’s impossible to say how these mutations impact the valuable compounds producers are interested in without performing more research.

“It’s an interesting piece of research, but it’s a preliminary study on a single plant,” says Igor Kovalchuk, a professor at the University of Lethbridge. “It’s kind of like if you took the DNA of an octogenarian and tried to guess the genotype of the human race.”

Documenting variation within a single plant is a solid first step for understanding how somatic mutations accumulate in cannabis plants, but it doesn’t give a ton of insight into how those mutations impact the important products a plant produces.

Both Kovalchuk and Desgagné-Penix suggest that an interesting next step would be taking a broader swath of genetic material, preferably from several generations of clonally propagated plants, to see how cannabinoid and terpene production is impacted over time. (In fact, Torkamaneh’s team is in the midst of testing terpene and cannabinoid production now.) An inter-generational study would provide greater insights into how long producers can actually maintain a mother plant before they need to change their stock. It’s an important point of management since many commercial practices dedicate about 15% of their valuable greenhouse space to maintaining mother plants, according to Torkamaneh.

Then, scientists could offer the burgeoning field more concrete advice on how to better manage cannabis mother plants to prevent changes in their output. In this way,

like the mosaicist of old, researchers can begin to piece together the landscape of cannabis mutations into one cohesive picture.

Dig deeper

Read the original article, "Accumulation of Somatic Mutations Leads to Genetic Mosaicism in Cannabis," here: **https://doi.org/10.1002/tpg2.20169**. Interested in cannabis? You may want to check out the recent article in *Crops & Soils* magazine providing crop advisers and producers with practical information and tips for growing cannabis in Canada: **https://doi.org/10.1002/crso.20146**.

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