University of Vermont Extension & Crops & Soils Program: , 2/20/20: 2nd Annual Industrial Hemp Conference

The science of cannabis: Looking under the hood of the cannabis plant



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https://youtu.be/BKWdcjCsHnw

Connecticut magazine

The professor

ERKOWITZ'S COURSE ATTRACTED 400 students, he tells me in late February, "but it's a pretty rigorous course, so we have about 300 left."

It's a safe bet that some of those students are looking to grow the psychoactive weed themselves, at home.



about cannabis," he says. "That's terrible that there isn't institutional scholarship going on."

And he's sending students to work in the industry in Connecticut. It's expanding, but it's not





https://youtu.be/BKWdcjCsHnw

UConn **Today**

BUSINESS CAMPUS LIFE

Cannabis Course Responds to Industry Need <

October 15, 2018 - Jessica McBride - Office of the Vice President for Research

EDUCATION

Cannabis 101 At The University Of Connecticut

March 16, 2019 · 1:59 PM ET Heard on All Things Considered



UConn To Offer Cannabis Course, But It's Not About Getting High



The Human Endocannabinoid System

CBD

20 Jun CBN is known to "fit" like lock and key into a network of existing receptors. The Endocannabinoid System exists to receive cannabinoids produced inside the body called "Anadnamine" and "2-Arachidonylglycerol." Stimulating the ECS with plant-based cannabinoids restores balance and helps maintain symptoms

> Cannabinoid Receptor

> > aitters

Presynaptic (sending neuron) CB1 receptors are concentrated in the brain and central nervous system but also sparsely populates other parts of the human body.

CB1

CBD does not directly "fit" CB1 or CB2 receptors but has powerful indirect effects still being studied.

CB2 receptors are mostly in the perepheral organs especially cells associated with the immune system.

CB2

Receptors are found on cell surfaces

Cannabidiol (CBD)

It is intriguing to consider that CBD does not 'make you high'; it is referred to as 'not psychotropic'. It is thought that CBD acts in the immune system and perhaps some other organs besides the brain. However, we know it affects seizures, by acting in the brain, so it must be doing something psychotropic. We do not know how CBD affects our bodies. Some research has suggested that CBD acts by binding to the following:



DEPARTMENT OF AGRICULTURE

[Doc. No. AMS-SC-19-0042; SC19-990-2 IR]

Establishment of a Domestic Hemp Production Program

as defined in the 2018 Farm Bill, the term "hemp" means the plant species Cannabis sativa L. and any part of that plant, including the seeds thereof and all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers, whether growing or not, with a delta-9 tetrahydrocannabinol concentration of not more than 0.3 percent on a dry weight basis. Delta-9 tetrahydrocannabinol, or THC, is the primary intoxicating component of cannabis. Cannabis with a THC level exceeding 0.3 percent is considered marijuana, which remains classified as a schedule I controlled substance

Where, for goodness sake, did the standard of 0.3% THC by weight come from?



CSA established 1970

Federal Controlled Substances Schedules

Schedule I (e.g. heroin, marijuana)

- A) High potential for abuse
- B) No currently accepted medical uses
- C) Lack of accepted safety for medical use

Schedule II (e.g. cocaine, methamphetamine)

- A) High potential for abuse Oxycontin
- B) Currently accepted medical use
- C) Potential for severe dependence

Schedule III (e.g. hydrocodone)

Steroids

- A) Lower potential for abuse than I and II
- B) Currently accepted medical use
- C) Potential for moderate or low dependence

Schedule IV (e.g. benzodiazepines) Valium

- A) Low potential for abuse relative to III
- B) Currently accepted medical use
- C) Potential for limited dependence relative to III

Schedule V (e.g. cough medicines w/ codeine)

- A) Low potential for abuse relative to IV
- B) Currently accepted medical use
- C) Potential for limited dependence relative to IV

Where, for goodness sake, did the standard of 0.3% THC by weight come from? TAXON 25(4): 405-435. AUGUST 1976

A PRACTICAL AND NATURAL TAXONOMY FOR CANNABIS*

Ernest Small** and Arthur Cronquist***

** Biosystematics Research Institute, Agriculture Canada, Ottawa, Canada K1A OC6. *** New York Botanical Garden, Bronx, N.Y. 10458.



It will be noted that we arbitrarily adopt a concentration of $0.3^{0/0}$ \triangle ⁹-THC (dry weight basis) in young, vigorous leaves of relatively mature plants as a guide to discriminating two classes of plants. This is based on standard-grown material in Ottawa in gardens, greenhouses and growth chambers, and of course on our analytical techniques. Dr. C. E. Turner, who has conducted extensive chemical analysis of Cannabis at the University of Mississippi, has agreed (pers. com.) that this is a reasonable figure to discriminate two classes of plants. We found that generally approximately 2% of the dry weight of young leaves of mature plants, or of the average dry weight of the softer parts of the female flowering plant (leaves, small twigs, flowers) is comprised of cannabinoids. Since CBD (cannabidiol, the most common non-intoxicant cannabinoid) and THC collectively usually compose the bulk of the cannabinoids present, one can crudely adjust literature reports of cannabinoid concentration for comparison with our values on the basis that the concentration of CBD and THC should sum to roughly $2^{0}/_{0}$.

Field 'Pharming' growing Cannabis as a crop for CBD extraction

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il and it

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Reddit post by a UConn student: 'Susan Herbst's private stash'

Cannabinoid synthesis involves shuttling of carbon skeletons from the monoterpene biosynthetic pathway to condense with hexanoyl CoA to generate olivetolic acid.





	Ca	annabis	gene nai	Motif function:		
	(numbers					
	TFX C	BDAS	GOT	PKS	OAS	(HORMONE):
SA responsive	1	1	2	1		Salicylic acid
XXX	10		2			
						ABA (abscisic
ABRE	2		1		6	acid)
MYB	5	6	3	6	5	~
MYC		1	1	1	2	
GA RE	3				1	Gibberilin 🛛 💽 🦂
auxin RE		1				Auxin

TFX is the propietary trichome-activating transcription factor CBDAS is CBDa synthase GOT is GPP:olivetolic acid transferase forms CBG (also referred to as prenyltransferase) PKS is polyketide synthase (forms Olivetolic acid) OAS olivetolic acid synthase (forms Olivetolic acid) Application of the growth regulator ethylene to Cannabis increases flower size, CBDaS expression, and female flower CBD content.



CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) has opened new era in biotechnology.

Provides simple, easy, cost effective and efficient access to manipulate virtually any part of the genome of any organism.



We can use CRISPR to edit many commercially-important Cannabis genes

RESEARCH

Open Access

The draft genome and transcriptome of *Cannabis* sativa

Harm van Bakel¹, Jake M Stout^{2,3}, Atina G Cote¹, Carling M Tallon³, Andrew G Sharpe³, Timothy R Hughes^{1,4*} and Jonathan E Page^{2,3*}





CBDAS

10

Hemp 'Finola' Log2(RPKM)

15

Plant Cell Reports (2018) 37:759–773 https://doi.org/10.1007/s00299-018-2265-x

ORIGINAL ARTICLE



OsPKS2 is required for rice male fertility by participating in pollen wall formation







There are known genes that can be edited to induce male sterility

A flow diagram for plant tissue culture



Conventional 'genetic engineering' or transformation involves using a bacterium, *Agrobacterium tumefaciens*, to deliver genes to cells in the callus

Meristematic cells from an explant are cultured in vitro to generate callus





Cannabis leaf explants with callus growing at cut surfaces



Close up of leaf explants; callus grows at cut end of veins



Cotyledon explants: great callus formation but no organogenesis

Callus: a growing mass of unorganized meristematic cells. Callus cells in plants grow to cover a wound; we take advantage of this to culture *in vitro*

Agrobacterium-Mediated Transformation of Cannabis

Some progresses made up to now: We have produced GUSpositive shoots but the efficiency needs to be improved.



Dr. Yi Li, UConn Plant Transformation Center, unpublished

Nano-Mediated Delivery of GUS DNA into Cannabis

Some progresses made up to now: nanoparticles help delivery of dsDNA into nucleus – the GUS gene is expressed. However, the efficiency is very low and thus needs to be improved.





Dr. Yi Li, UConn Plant Transformation Center, unpublished

A significant problem for commercial hemp production: sometimes your hemp tests 'hot' for THC (>0.3%) as the flowers mature. What can be done to prevent THC production in hemp?

RATIO INHERITANCE

CBD to THC Ratio Inheritance: in this project to identify the heritability of specific CBDa and THCa synthases through self-pollination and outcrossing of "ultra high CBD" (>50:1 ratio) plant lines. The secondary goal of this project is to identify specific combinations of synthases that allow for very high cannabinoid content plants (>20% d.w.) to meet federal THC guidelines for hemp. Our in-house research program was the first to identify the cause of THCa production in type III (CBD) and type IV (CBG) varieties; this is due to the presence of multiple CBCA synthase gene copies.



Real-time quantitative PCR

- Different genes → different tissues
- Use qPCR to determine differential expression of genes
- THCa largely produced in flowers



What step in the biosynthetic pathway rate-limits cannabinoid production? Evaluation of Cannabinoid biosynthetic genes over the course of the flowering period in hemp

Hypothesis: CBDa synthase expression is correlated with CBDa production in hemp; THCa synthase is correlated with THCa production in marijuana

- took weekly, RNA samples during flowering cyclce
- Week 1 represents the onset of flowering; the first female flower parts (stigmata) appear
- Week 7 represents samples collected just prior to harvest



Week 1

Week 7

Hemp:



From Oregon CBD web page: The federal THC limit is 0.3% for industrial hemp. I've seen other seed companies claiming consistent results below that level. Will your seed produce a field of 0.3% THC plants? Answer: In short, yes—all of our type III (CBD) and type IV (CBG) industrial hemp varieties have their THC production synthases turned "off" through traditional <u>selective breeding</u> (non-GMO), with chemical and constitution autiding up

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Industrial Hemp Seed Research and Development





Hemp: other genes of interest



Correlation matrix

- Pearson's Correlation coefficient
- Blue = positive correlation
- Red = negative correlation
- 1 star P value of 0.05
- 2 stars P value of 0.01
- 3 stars P value of 0.001



Marijuana – THCa synthase

- Data did not support hypothesis
- Data do not suggest direct correlation between THCa synthase and its product
- Relative to week 1, there is negligible changes in gene expression throughout the entire period of flower development



Marijuana - GOT

- Data suggest a stronger correlation of THCa production with GOT gene expression levels
- Suggests that this enzyme, GOT, may be a more influential rate limiting factor in THCa production

Expression of GOT and CBGa content over flowering period



Marijuana – other genes of interest



Marijuana – CBDa synthase

- There are no statistically significant changes in relative expression of CBDa synthase
- Correspondingly, there is no detectable CBDa in the flower tissue at any point during development

Relative expression of CBDa synthase



Cannabinoids are produced in glandular trichomes, and trichomes are most abundant on unfertilized female flowers







A promoter is an area of DNA where transcription of the DNA begins. The promoter controls the expression of the gene by signaling to RNA polymerase to begin transcription and the direction in which the polymerase should Site Site



Specific genetic elements in the 'upstream' promoter region of a gene can bind regulatory proteins (<u>transcription factors</u>) and act to enhance or repress the ability of RNA polymerase to bind to, and transcribe the protein encoded by the genetic sequence of the coding region.



Typical gene organization



Trichome-related gene of interest

- Other plants that produce trichomes and have secondary metabolites have trichome specific genes
- Identified a homolog in *C. sativa* ٠
- The expression of this gene • corresponds with the increase in THCa generation



content 15 25 expression 20 10 conte 10 Relative 5 5 rHCa Weeks after short day intiation

TF-X — THCa content

Transcription factor X vs THCa

	(Cannabis ;	gene nar	ne	Motif function:	
(r	numbers					
	TFX	CBDAS	GOT	PKS	OAS	(HORMONE):
SA responsive	1	1	2	1		Salicylic acid
XXX	10		2			
						ABA (abscisic
ABRE	2		1		6	acid)
MYB	5	6	3	6	5	
MYC		1	1	1	2	
GA RE	3				1	Gibberilin 🛛 💽
auxin RE		1				Auxin 🔂

TFX is the propietary trichome-activating transcription factor CBDAS is CBDa synthase GOT is GPP:olivetolic acid transferase forms CBG (also referred to as prenyltransferase) PKS is polyketide synthase (forms Olivetolic acid) OAS olivetolic acid synthase (forms Olivetolic acid)

Marijuana

Found a statistically significant 3.3-fold increase in TF-X (P < 0.10)

Relative Expression of TF-X after application



Marijuana

THCa content in C. sativa plants after application

Observed a statistically significant 21% increase in cannabinoid content post-treatment (P < 0.05)



Pearson's Product-Moment Correlation Matrix evaluating the positive (
) and negative
(
) relationships between cannabinoids and biosynthetic pathway enzymes in medical
marijuana. Shade and size of circles denote extent of correlation. Stars indicate
significance (5, 1, and 0.1%) evaluated using ANOVA. Pearson's Product-Moment
Correlation was used to generate correlations.

What have we learned?

- Trichome initiation TF expression is correlated with THC and many of the cannabinoid synthesis genes.
- 2. Production of GPP may be a new target of breederslooking for increased cannabinoids as well as monoterpenes.







ORIGINAL RESEARCH published: 05 June 2018 doi: 10.3389/fpls.2018.00765

A Dual Repeat *Cis*-Element Determines Expression of *GERANYL DIPHOSPHATE SYNTHASE* for Monoterpene Production in *Phalaenopsis* Orchids



Conclusions

- Rate limiting factor influence may depend on the variety
 - GOT expression correlates with THCa production in C. sativa 'Gorilla Glue'
 - CBDa synthase and GOT expression seem to correlate equally well with CBDa production in C. sativa 'Cherry wine'
- Genes related to trichome development may be important for breeders as well

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