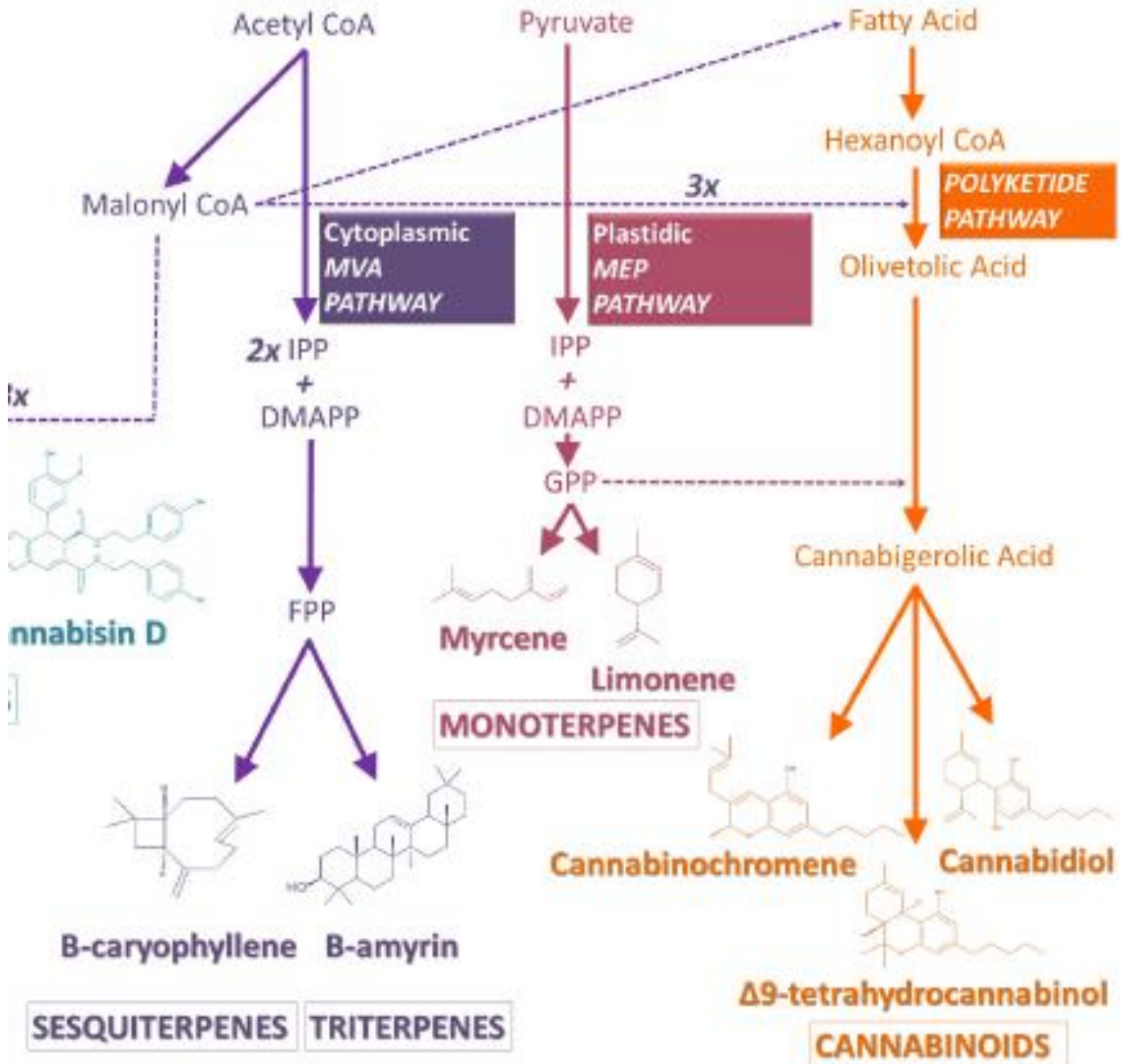
CT 2020 Hemp Conference:

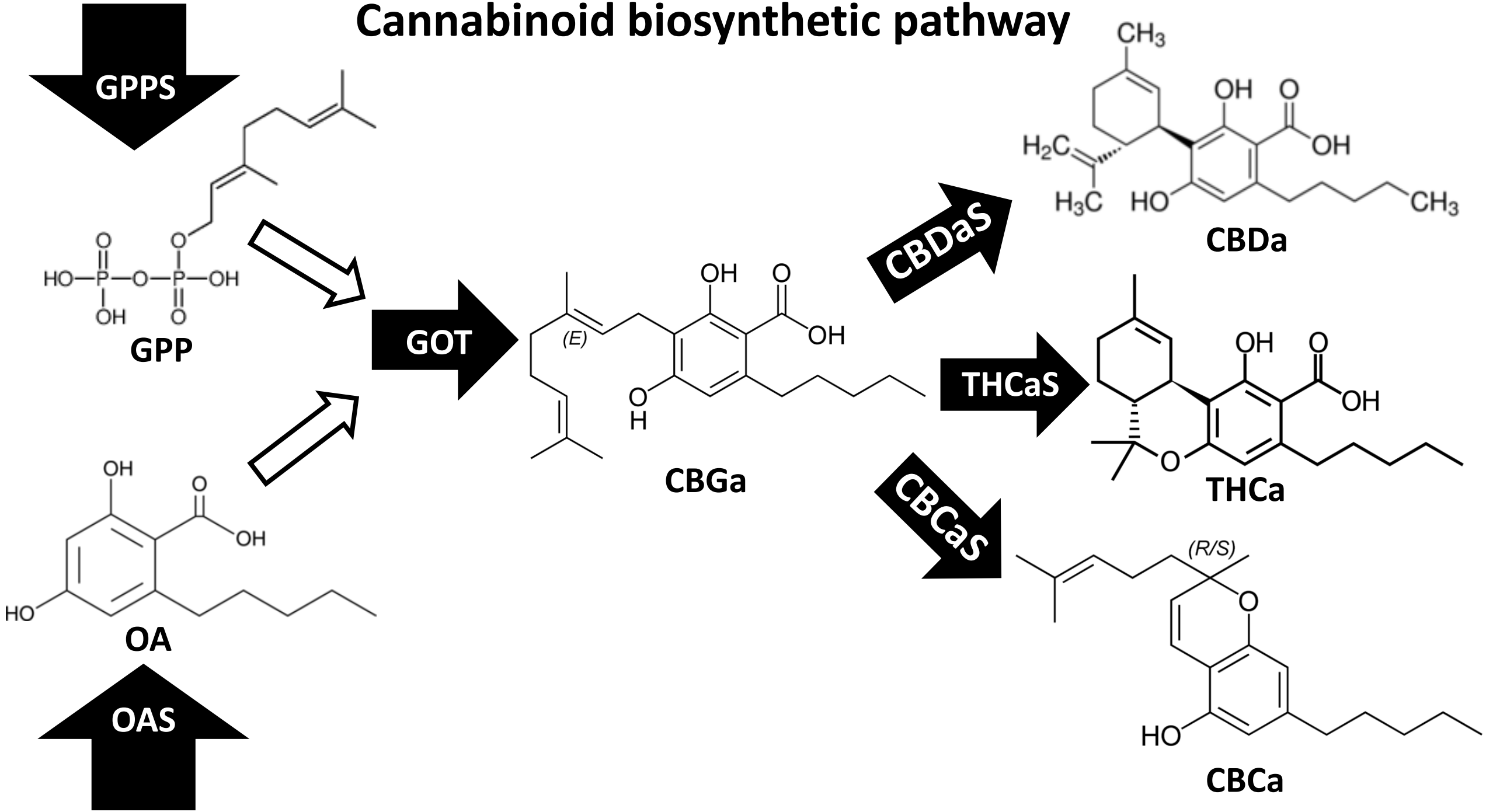
Looking Under the Hood of the Cannabis Plant: Molecular Studies of How to Increase End-Product (CBD and THC) Cannabinoid Synthesis

Peter Apicella, Yi Ma, and Gerald Berkowitz

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



Cannabinoid biosynthetic pathway



I. 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

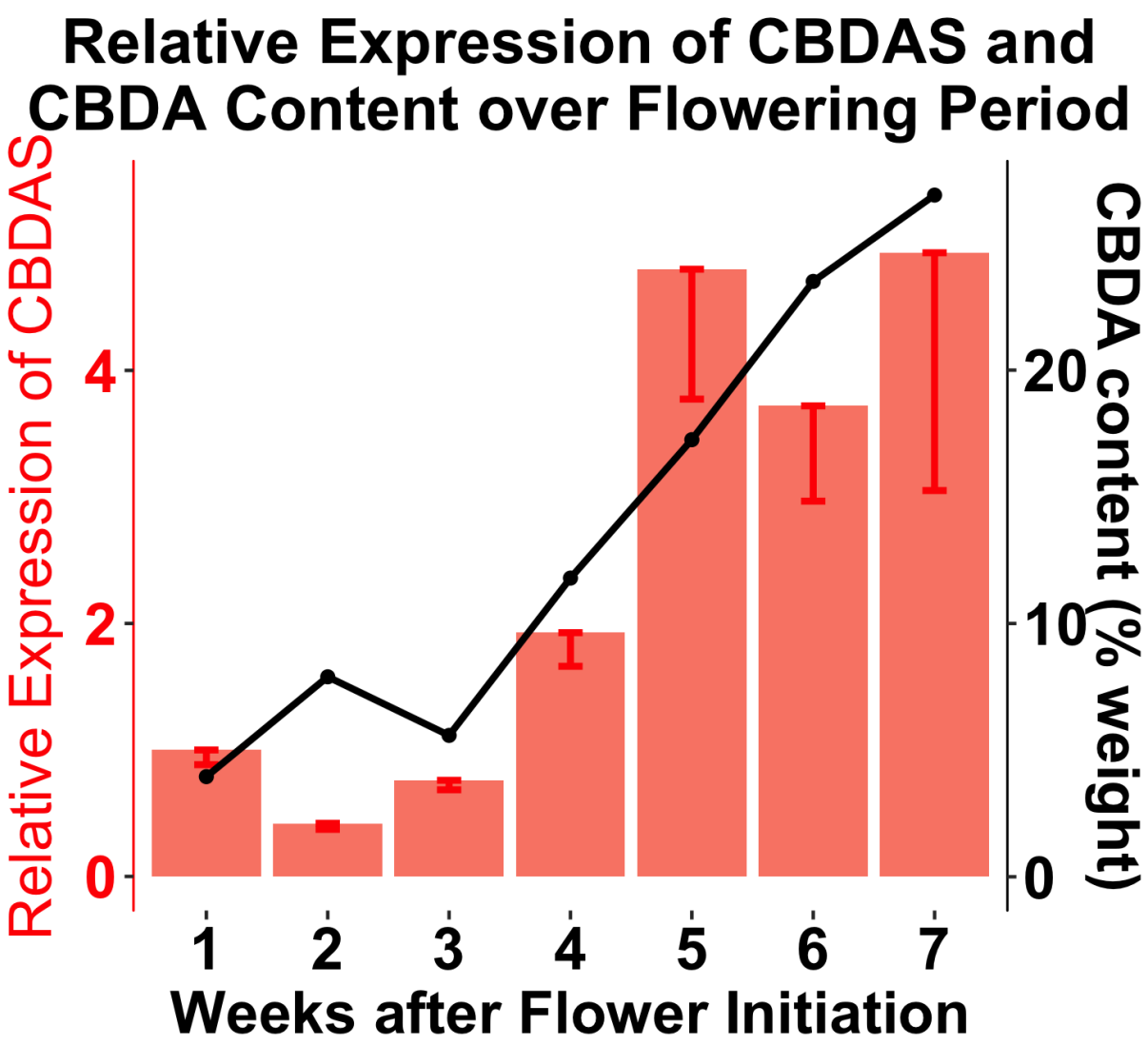
- took weekly, RNA samples during flowering cycle
- Week 1 represents the onset of flowering; the first female flower parts (stigmata) appear
- Week 7 represents samples collected just prior to harvest

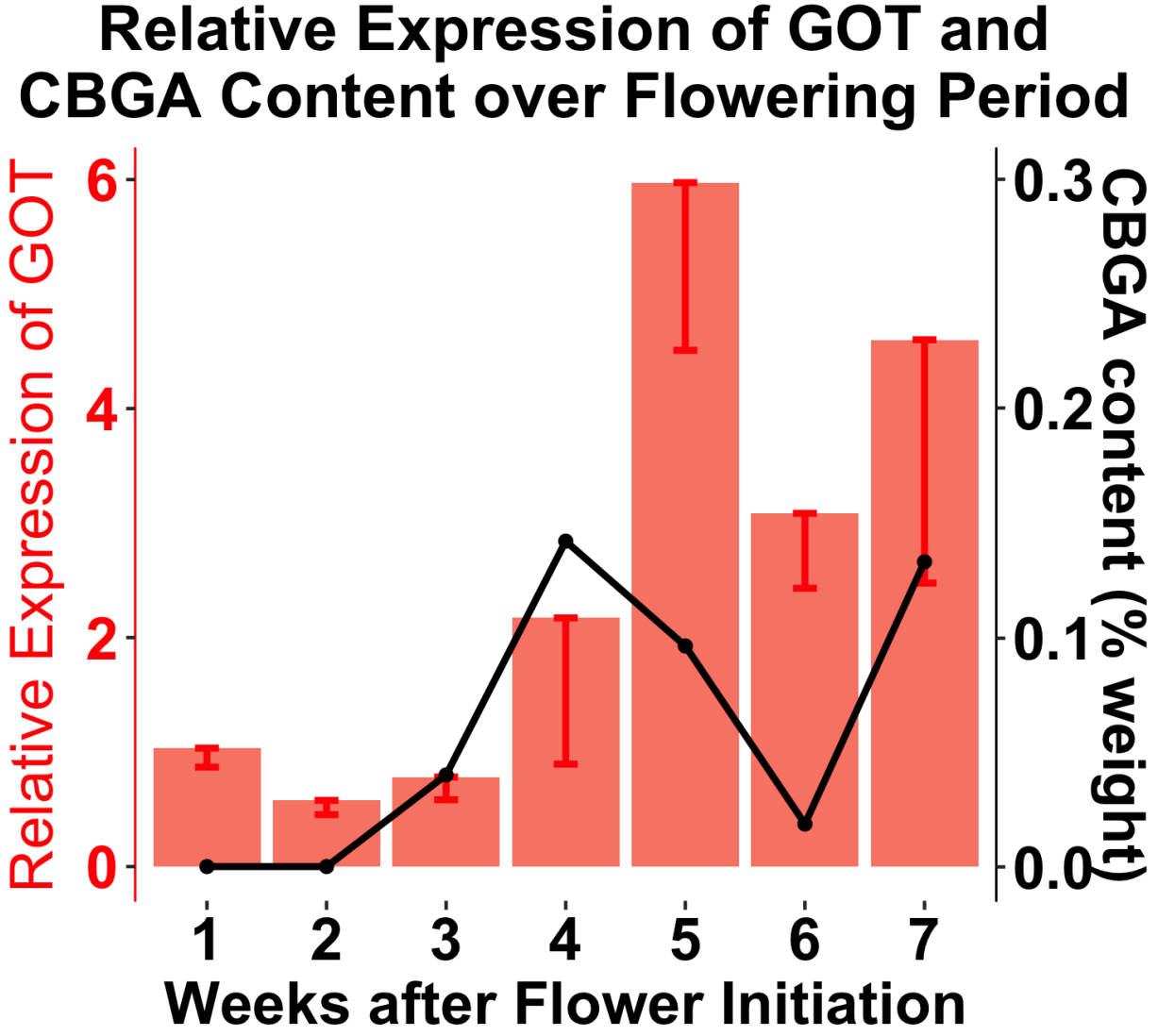
Week 1

Week 7

II. What gene product is responsible for THC production in commercial CBD hemp cultivars?

Hemp:

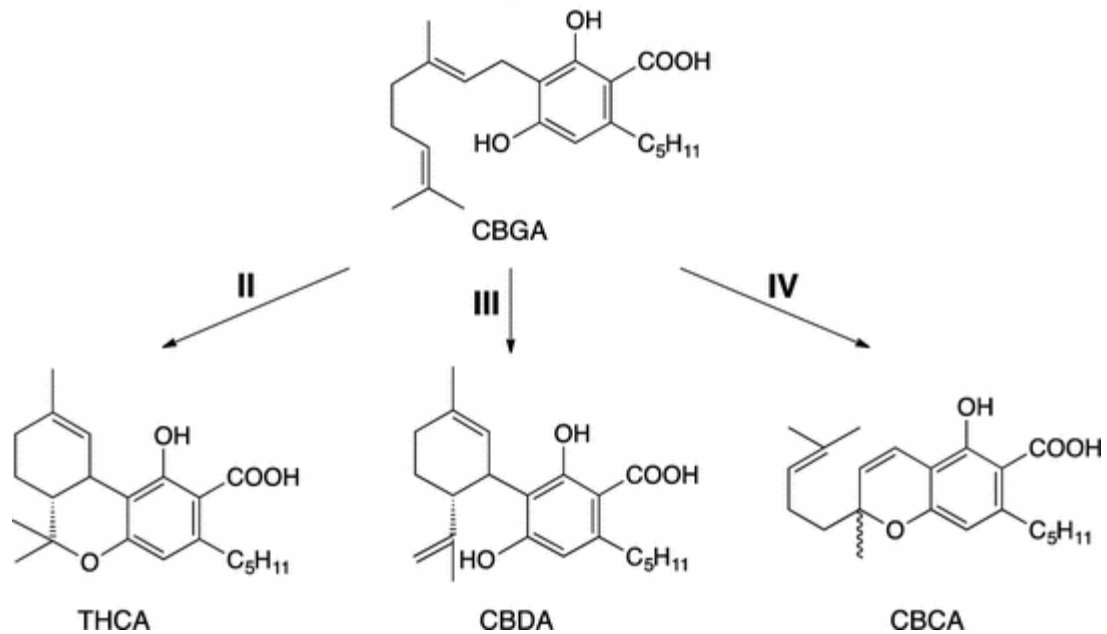

Relative Expression of CBDAS and CBDA Content over Flowering Period
Relative Expression of CBDAS
CBDA content (% weight)
0
2
4
0
10
20
1
2
3
4
5
6
7
Weeks after Flower Initiation


Relative Expression of GOT and CBGA Content over Flowering Period
Relative Expression of GOT
CBGA content (% weight)
0
2
4
6
0.0
0.1
0.2
0.3
1
2
3
4
5
6
7
Weeks after Flower Initiation

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?

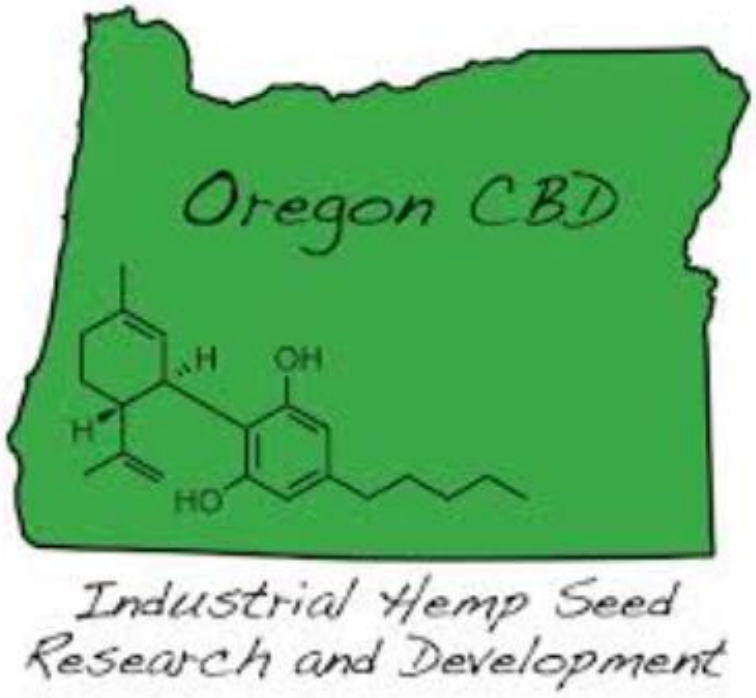
RATIO INHERITANCE

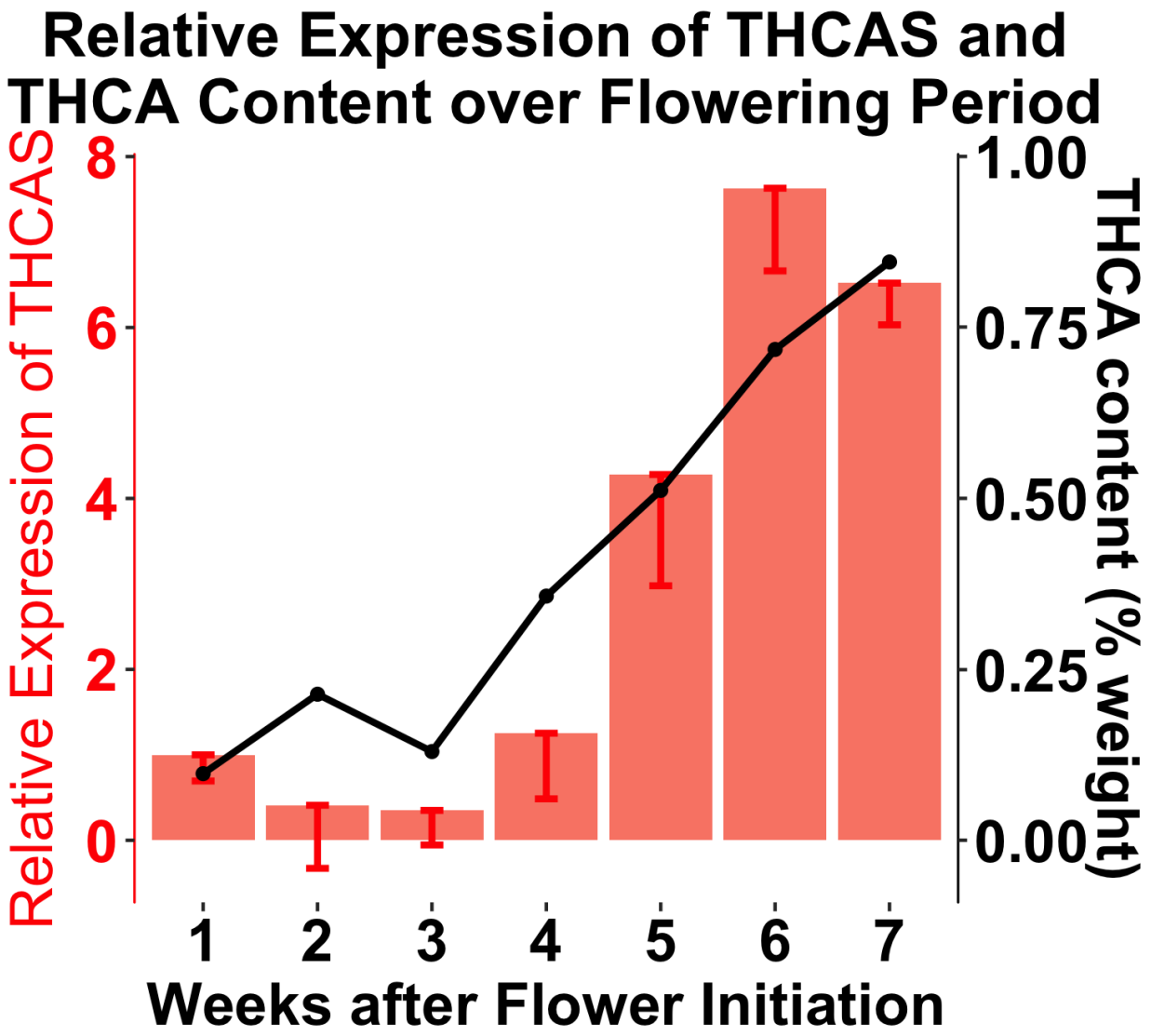
CBD to THC Ratio Inheritance: in this project to identify the heritability of specific CBDa and THCa synthases through self-pollination and outcrossing of “ultra high CBD” (>50:1 ratio) plant lines. The secondary goal of this project is to identify specific combinations of synthases that allow for very 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.**



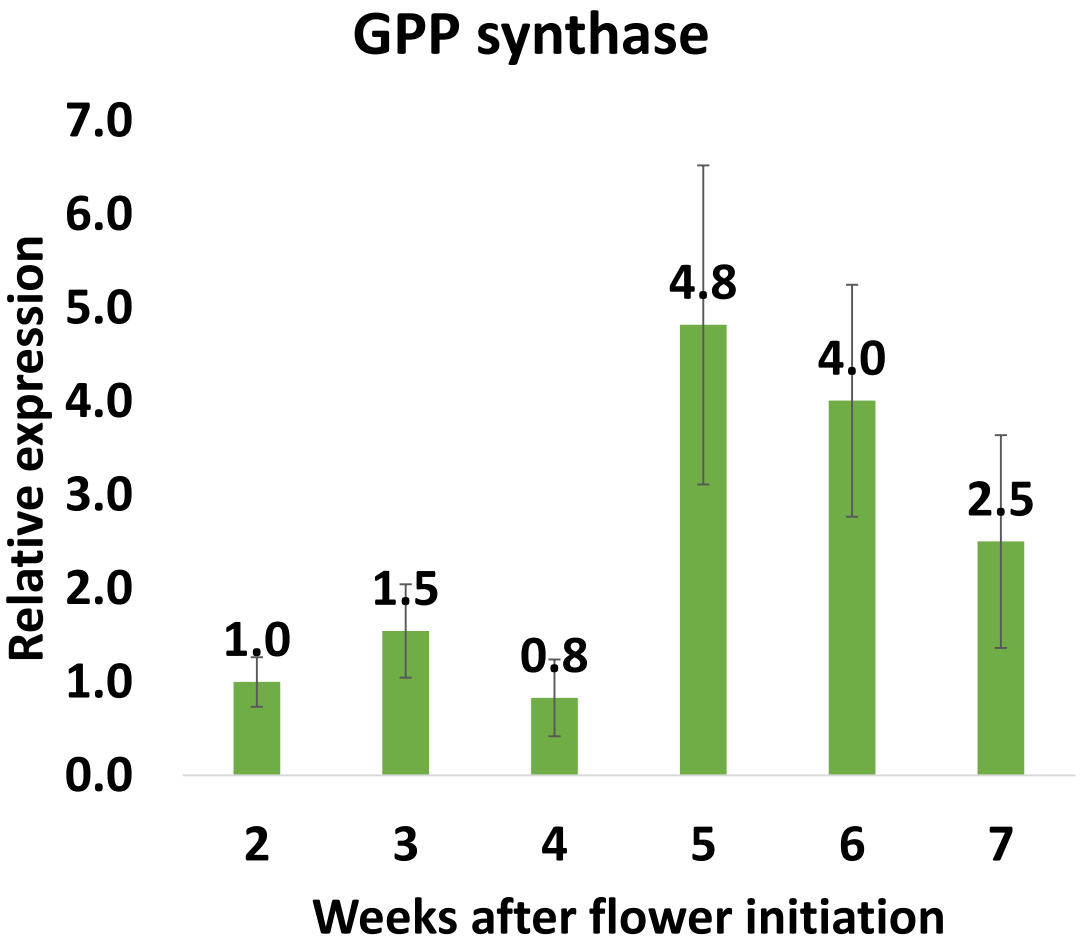
Hemp: Cherry Wine

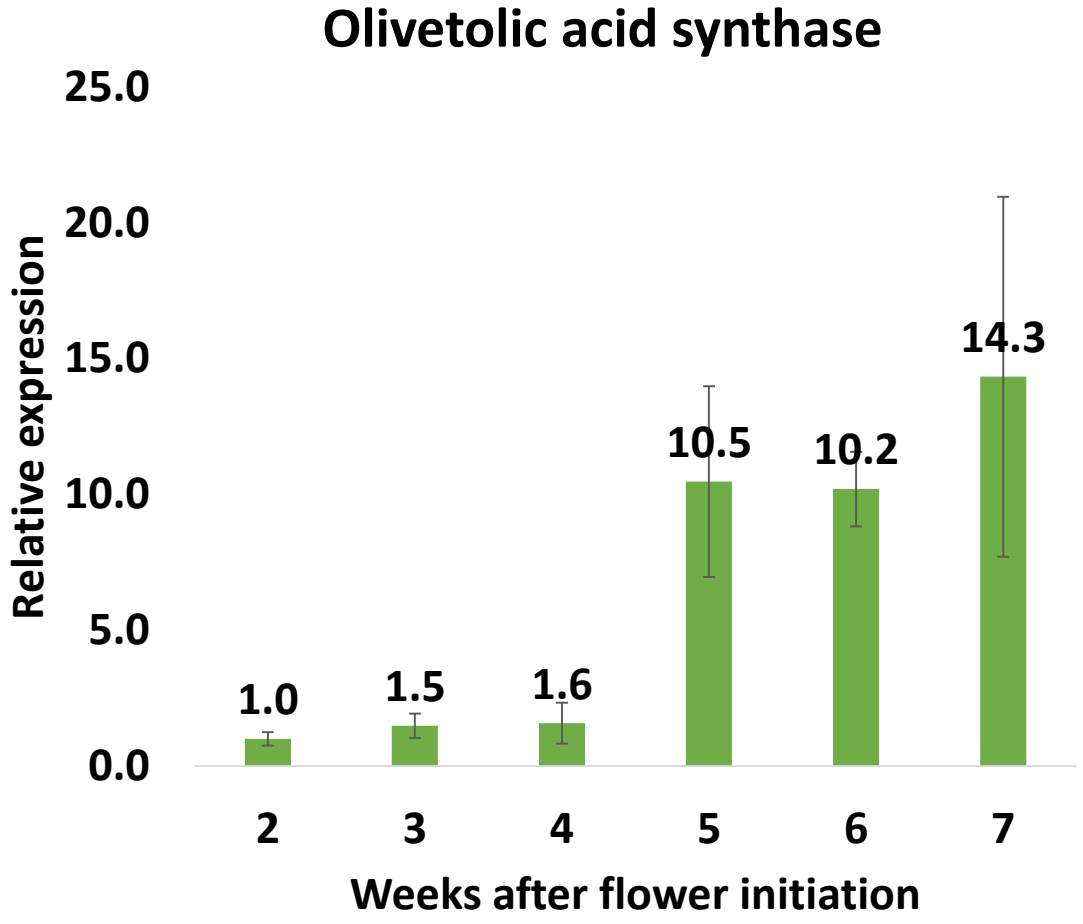
From Oregon CBD web page: The federal THC limit is 0.3% for industrial hemp. I've seen other seed companies claiming consistent results below that level. Will your seed produce a field of 0.3% THC plants? Answer: In short, yes—*all of our type III (CBD) and type IV (CBG) industrial hemp varieties have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





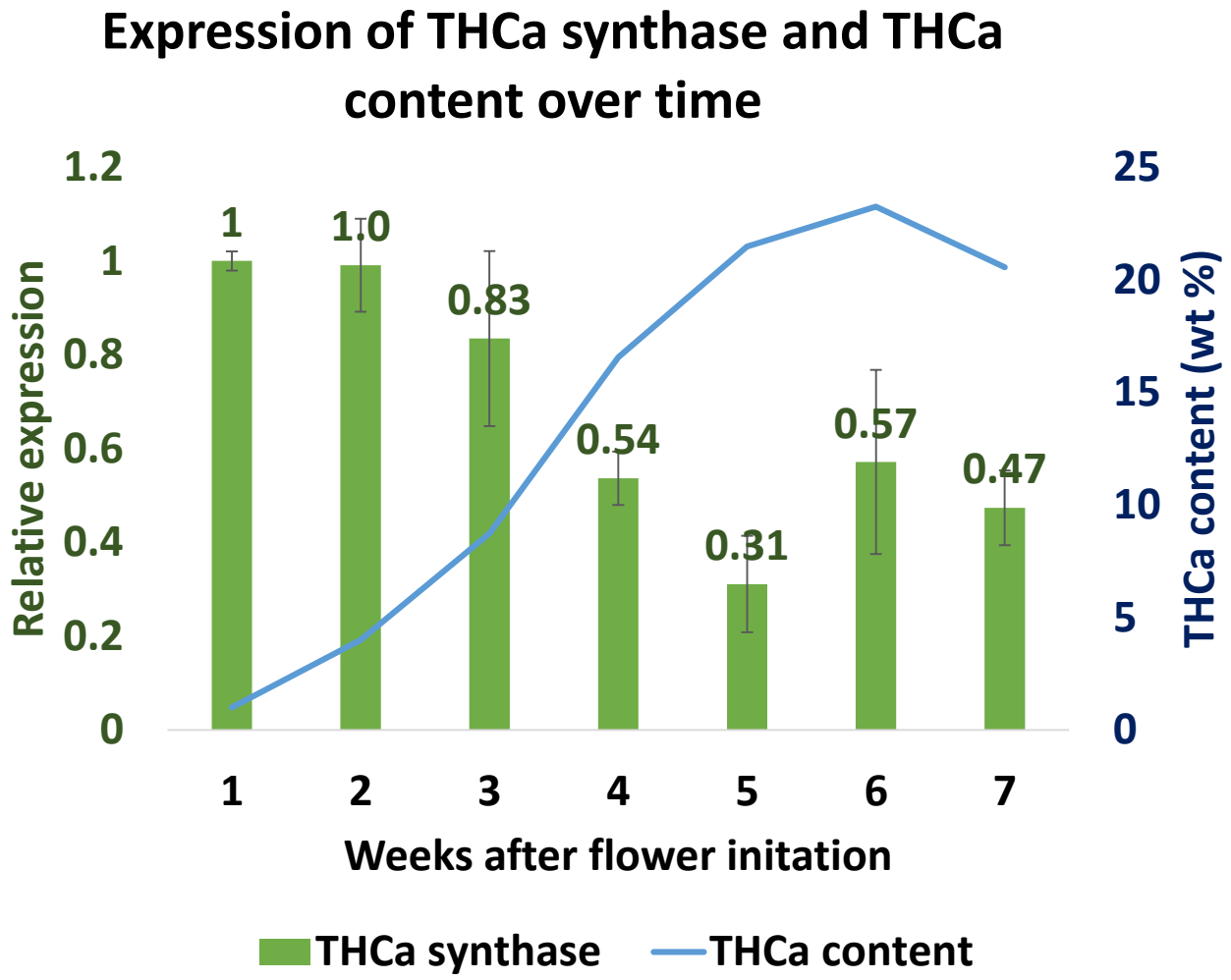
Hemp: other genes of interest





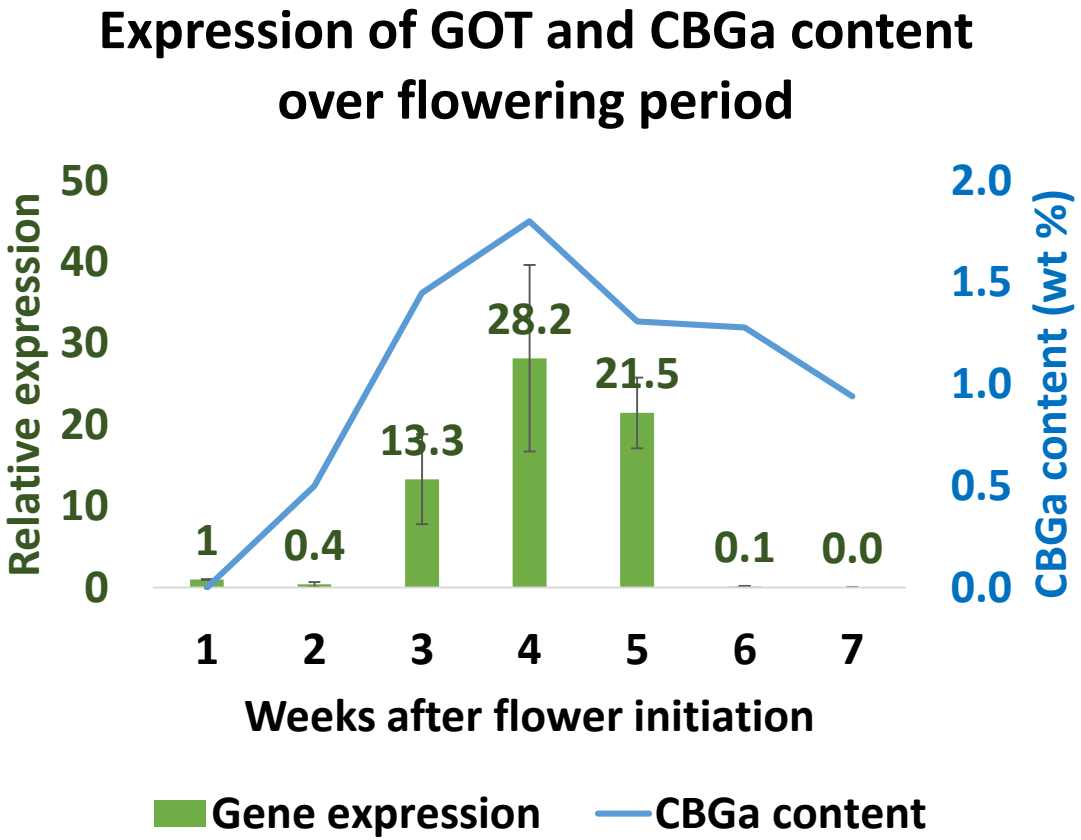
Marijuana – THCa synthase

- 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



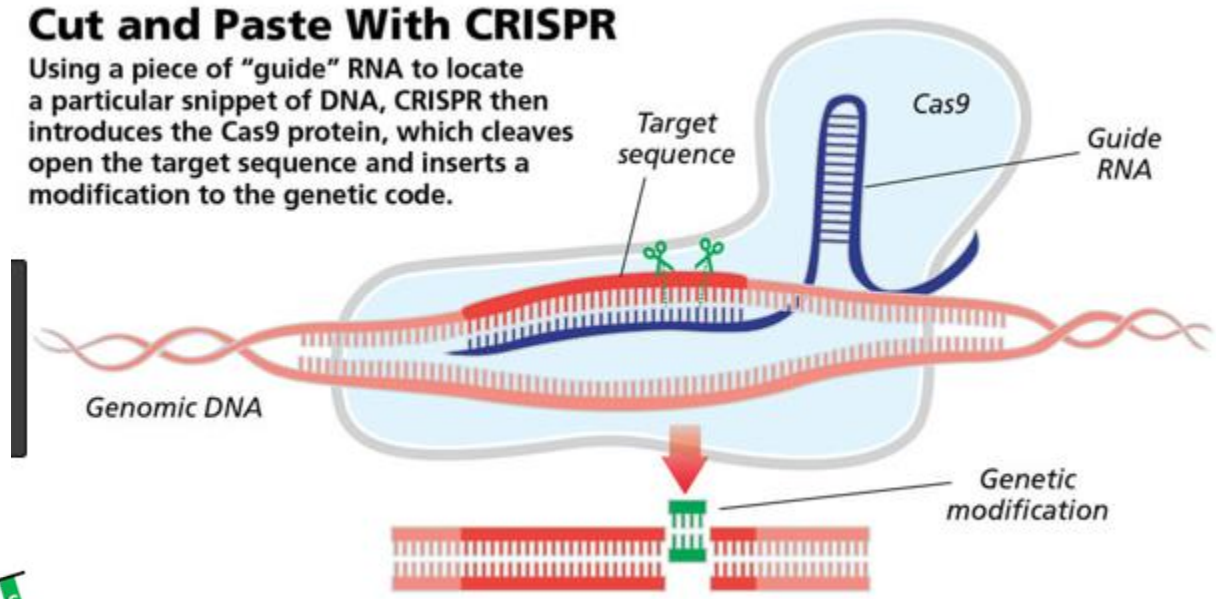
Marijuana - GOT

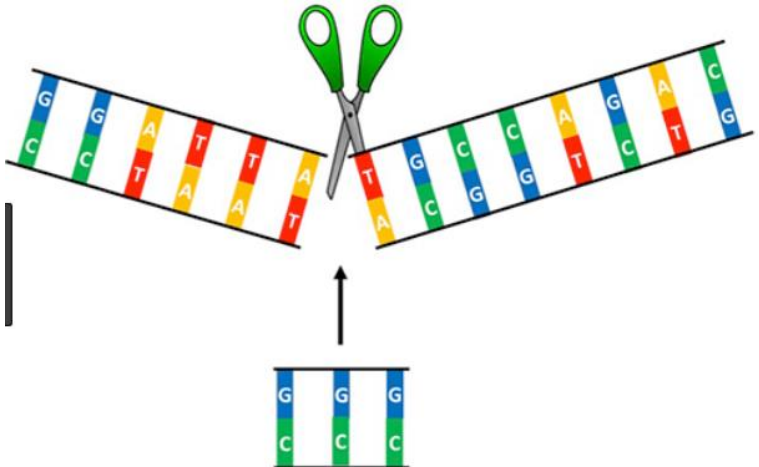
- Data suggest a stronger correlation of THCa production with GOT gene expression levels
- Suggests that this enzyme, GOT, may be a more influential rate limiting factor in THCa production



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

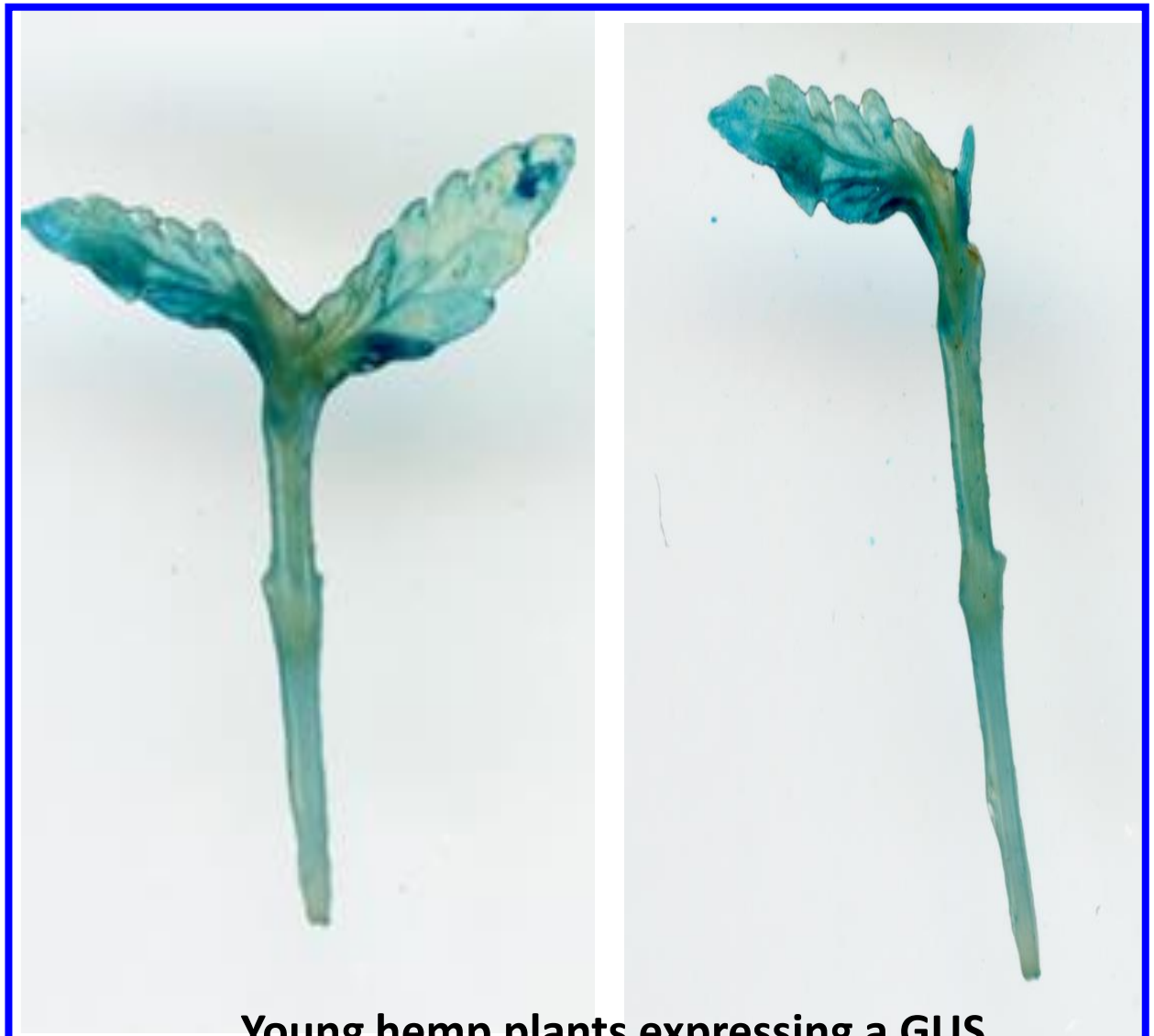
Provides simple, easy, cost effective and efficient access to manipulate virtually any part of the genome of any organism.





CRISPR/Cas9 technology is a recently developed technology. In some cases, it can be used to make genetic changes in crop plants that will **not** result in GMO plants according to a recent USDA determination

Genetic Transformation of Hemp/Cannabis Plants



Young hemp plants expressing a GUS reporter gene (blue color)

Yi Li Laboratory at the University of Connecticut has developed an efficient genetic transformation method for hemp/cannabis plants.

It is now possible to use gene editing and other modern tools to create desirable traits for hemp/cannabis plants.

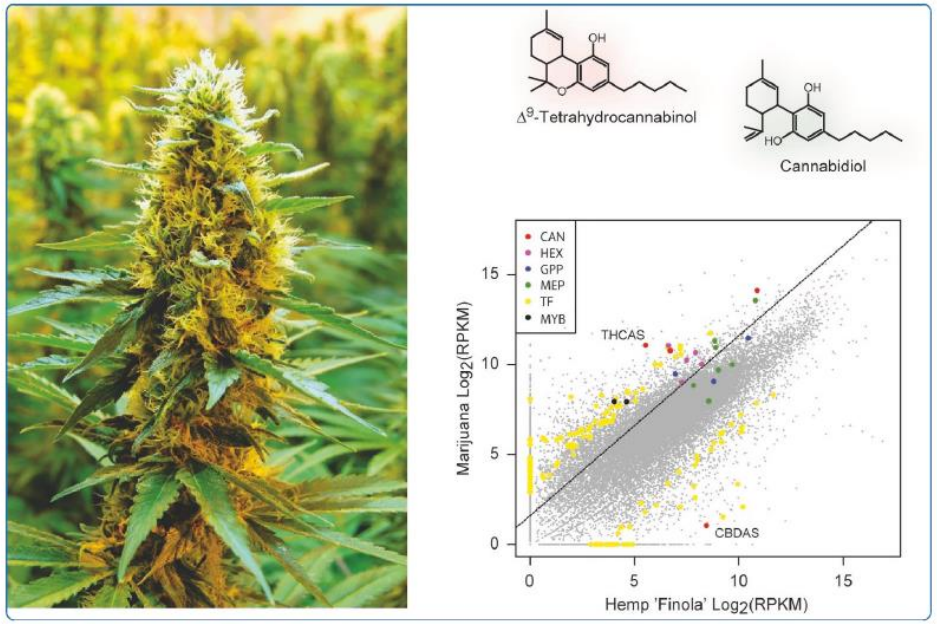
We can use CRISPR to edit many commercially-important Cannabis genes

RESEARCH Open Access

The draft genome and transcriptome of *Cannabis sativa*

Harm van Bakel[1], Jake M Stout[2,3], Atina G Cote[1], Carling M Tallon[3], Andrew G Sharpe[3], Timothy R Hughes[1,4*] and Jonathan E Page[2,3*]

Genome Biology

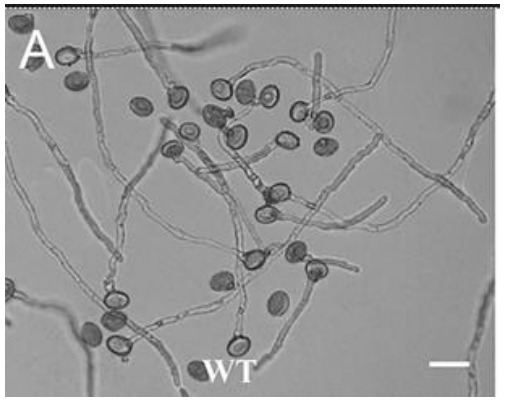


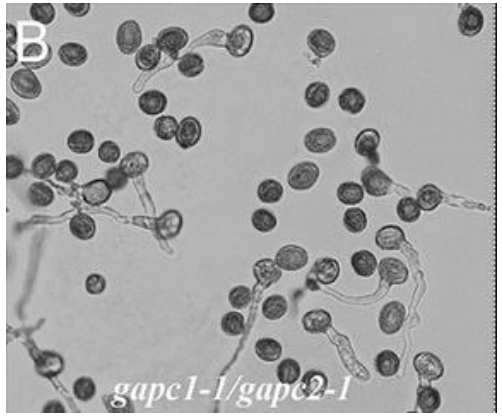
Plant Cell Reports (2018) 37:759–773
https://doi.org/10.1007/s00299-018-2265-x

ORIGINAL ARTICLE

CrossMark

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

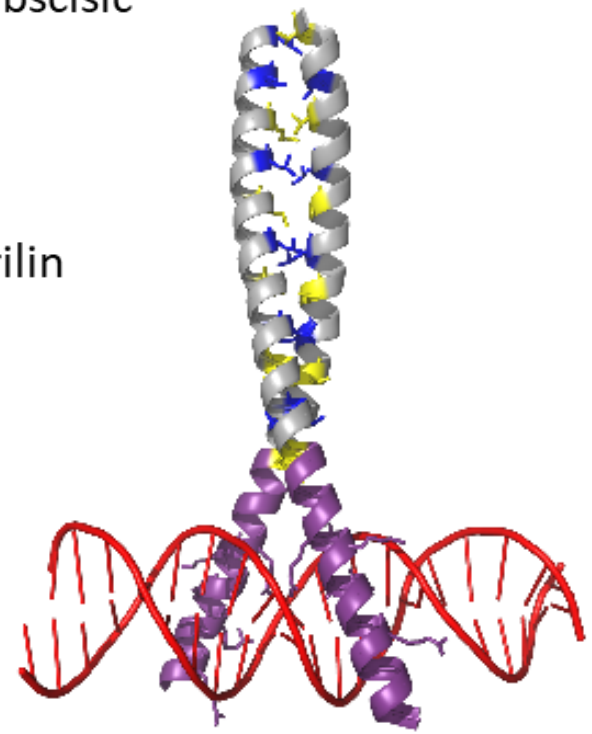




There are known genes that can be edited to induce male sterility

III. What factors regulate expression of cannabinoid biosynthetic pathway genes?

	Cannabis gene name (numbers are amount of motifs in genes)					Motif function:
	TFX	CBDAS	GOT	PKS	OAS	(HORMONE):
SA responsive	1	1	2	1		Salicylic acid
XXX	10		2			
ABRE	2		1		6	ABA (abscisic 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)

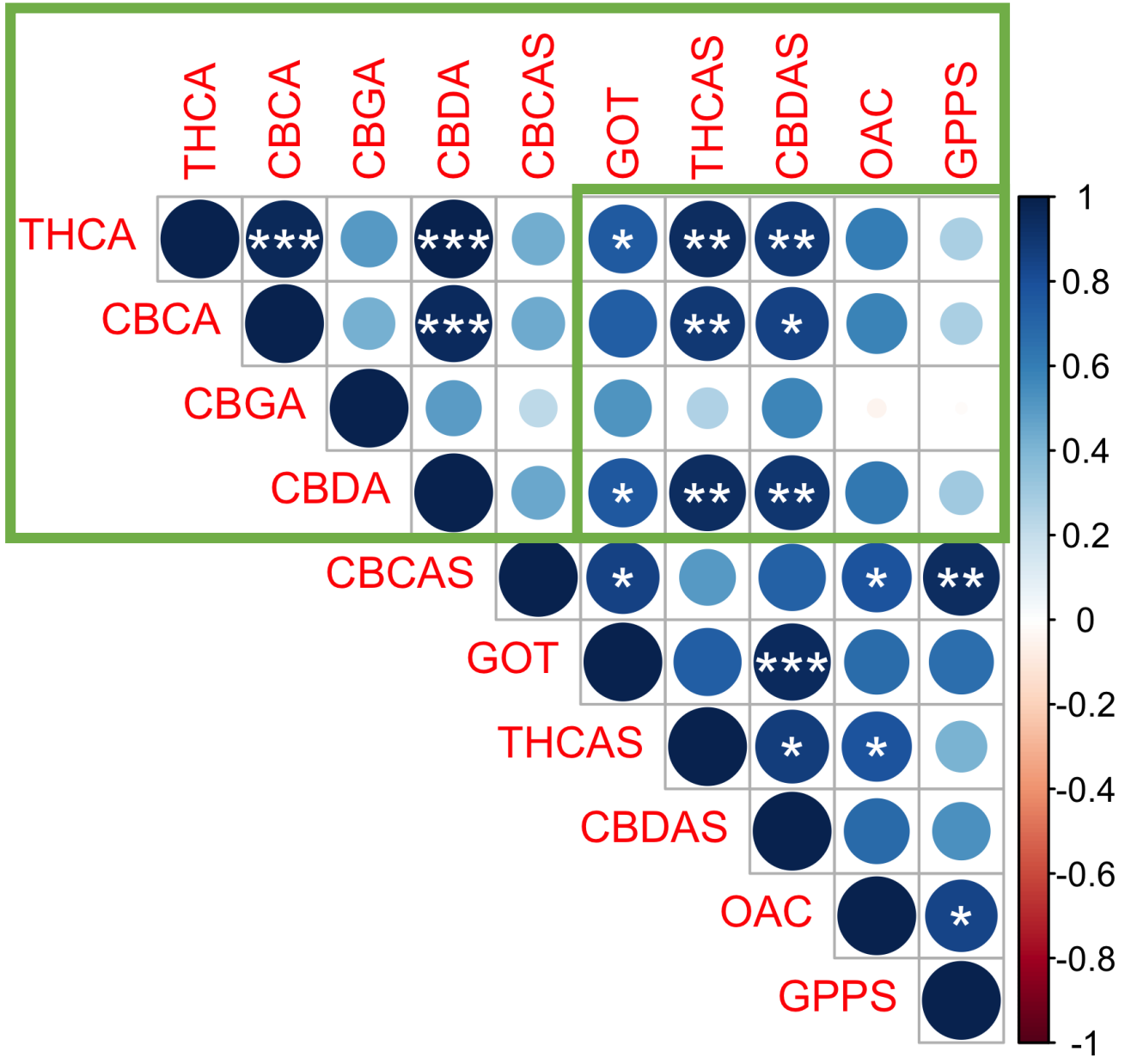
PKS is polyketide synthase (forms Olivetolic acid)

OAS olivetolic acid synthase (forms Olivetolic acid)

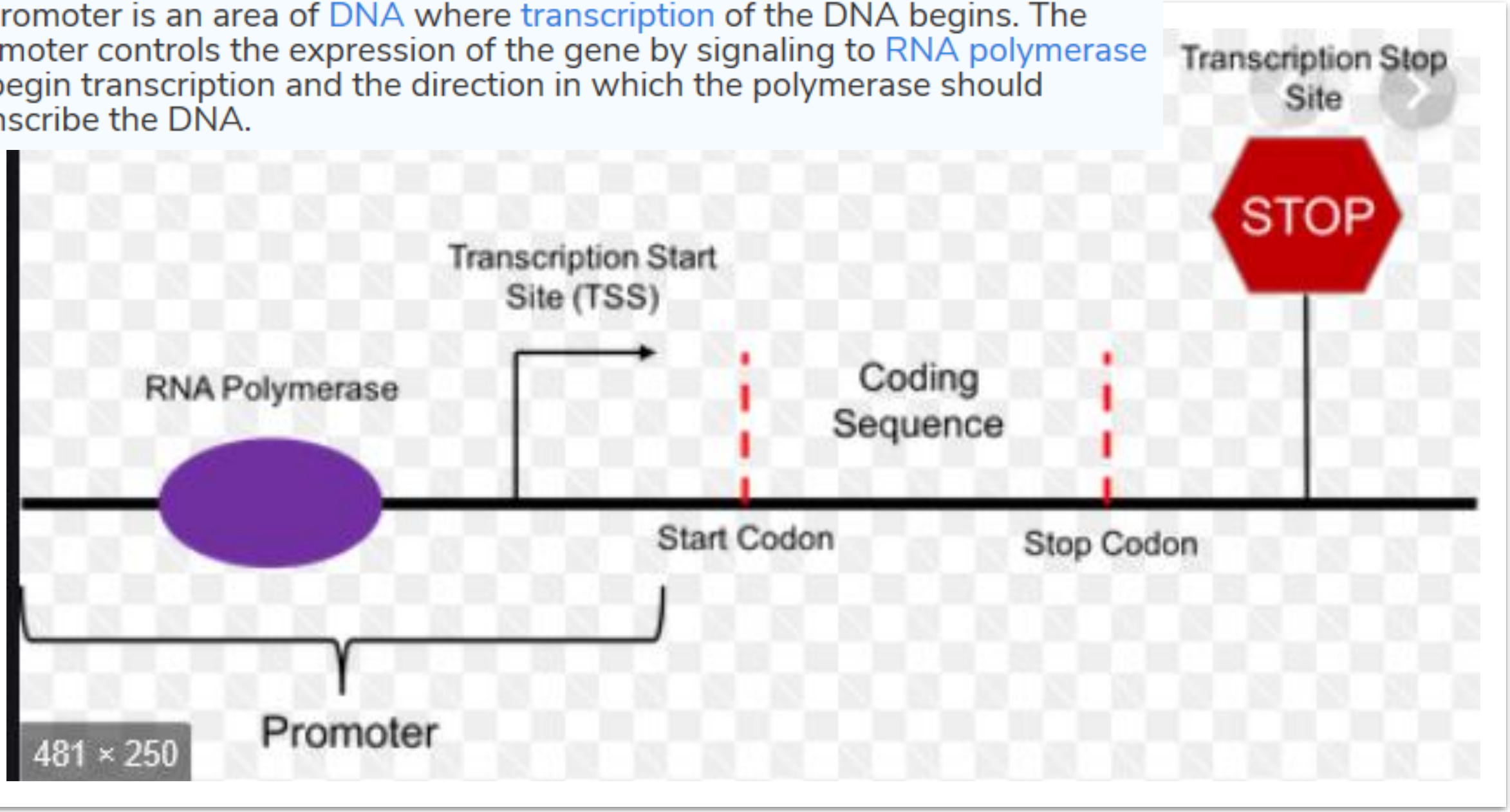
Correlation matrix

- Pearson's Correlation coefficient
- **Blue** = **positive correlation**
- **Red** = **negative correlation**

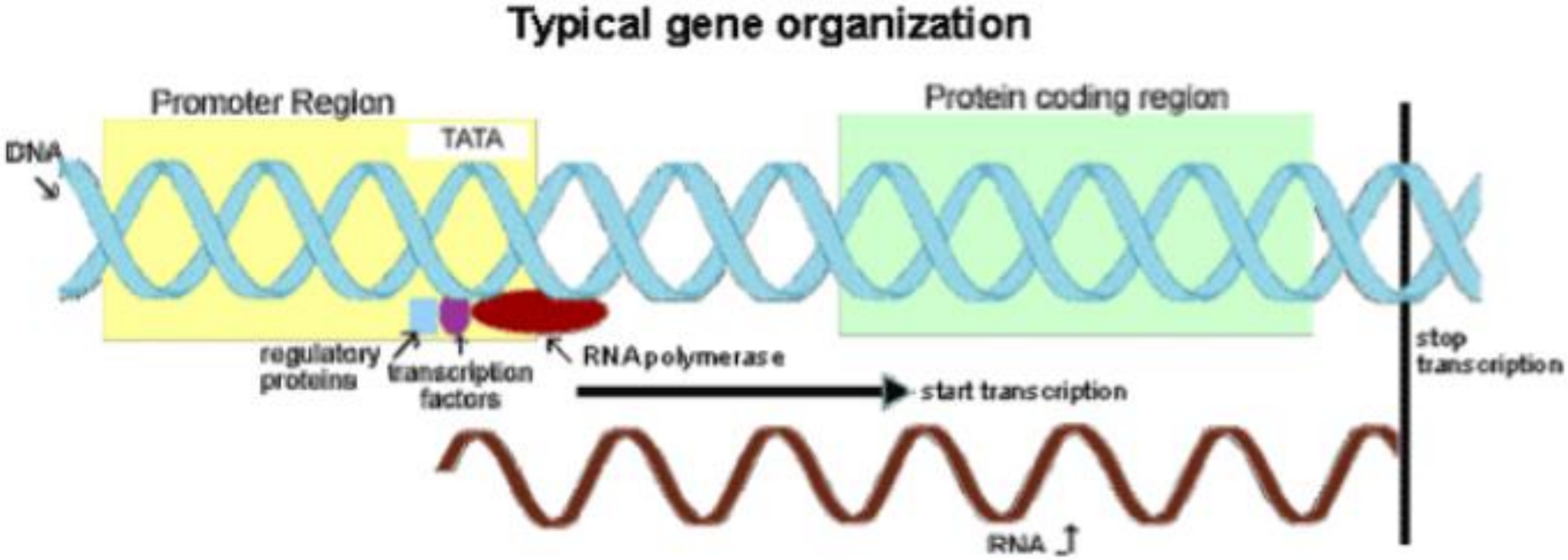
- 1 star - P value of 0.05
- 2 stars – P value of 0.01
- 3 stars - P value of 0.001



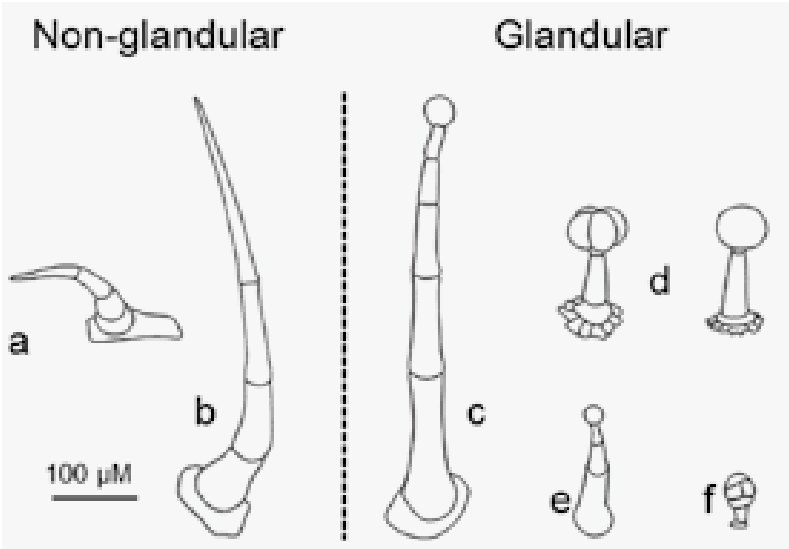
A promoter is an area of DNA where transcription of the DNA begins. The promoter controls the expression of the gene by signaling to RNA polymerase to begin transcription and the direction in which the polymerase should transcribe the DNA.



Specific genetic elements in the ‘upstream’ promoter region of a gene can bind regulatory proteins (**transcription factors**) 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.

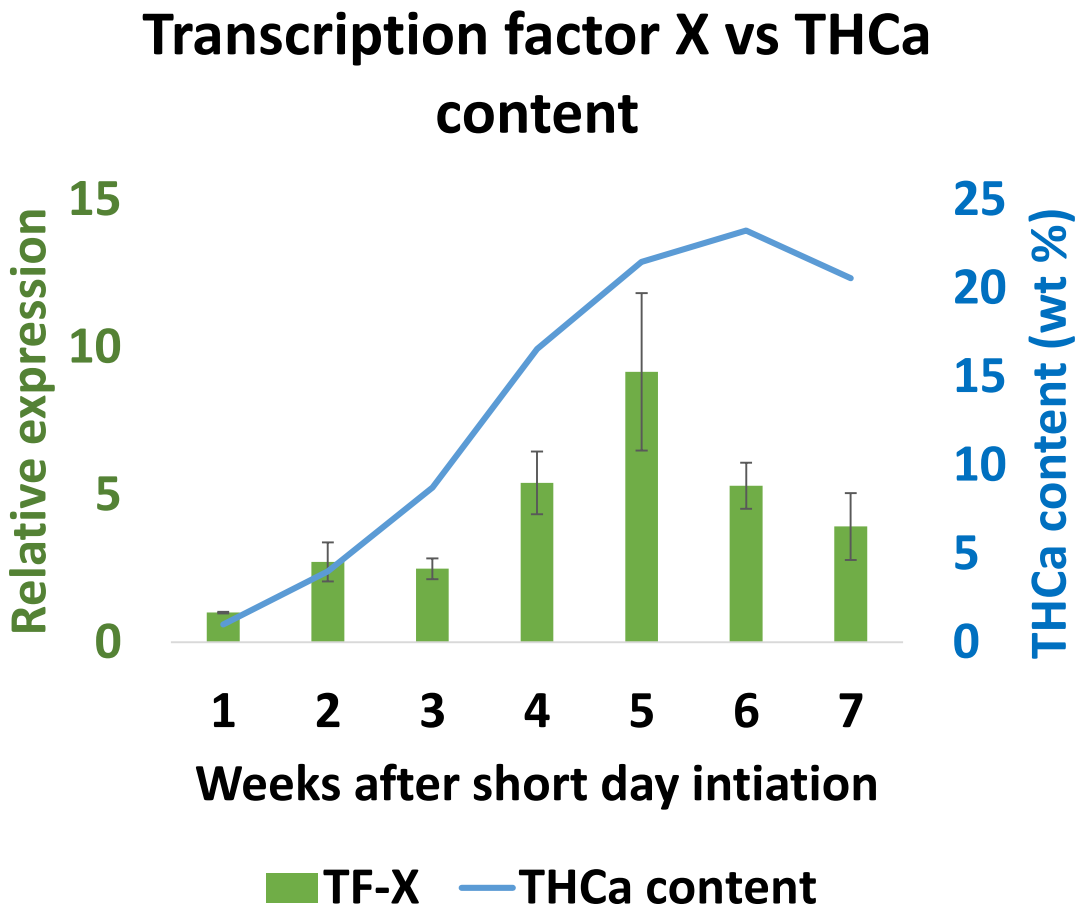


Cannabinoids are produced in glandular trichomes, and trichomes are most abundant on unfertilized female flowers

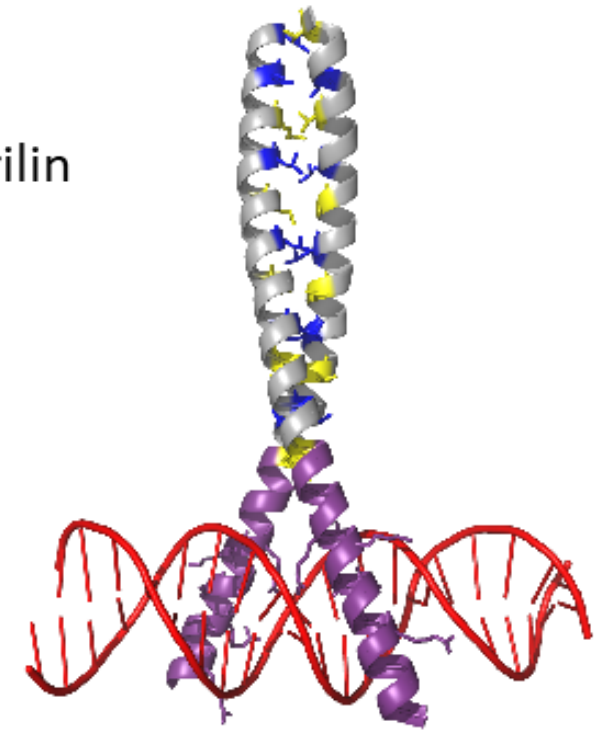


Trichome-related gene of interest

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



	Cannabis gene name (numbers are amount of motifs in genes)					Motif function:
	TFX	CBDAS	GOT	PKS	OAS	(HORMONE):
SA responsive	1	1	2	1		Salicylic acid
XXX	10		2			
						ABA (abscisic acid)
ABRE	2		1		6	
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

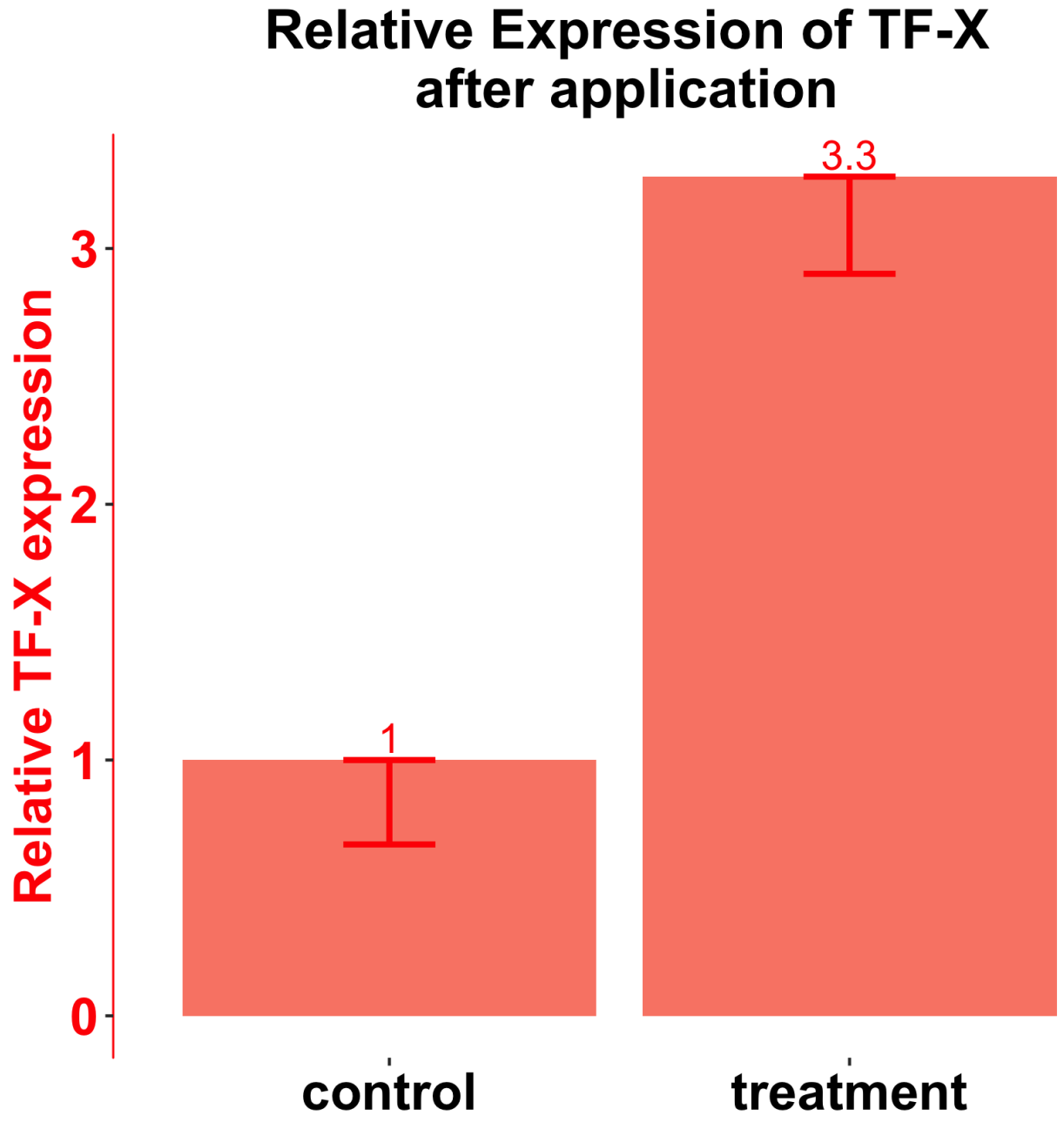
GOT is GPP:olivetolic acid transferase forms CBG (also referred to as prenyltransferase)

PKS is polyketide synthase (forms Olivetolic acid)

OAS olivetolic acid synthase (forms Olivetolic acid)

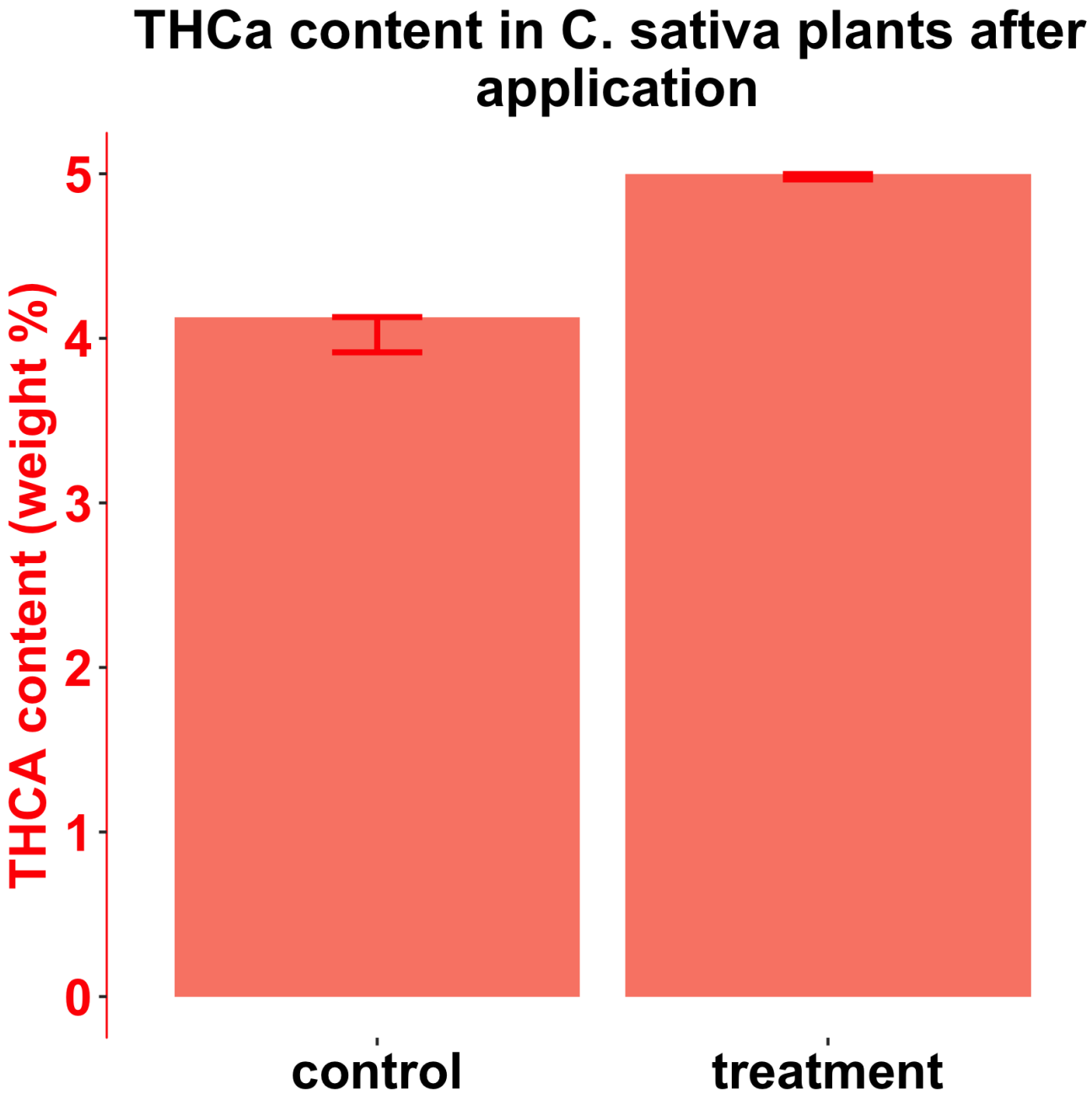
Marijuana

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



Marijuana

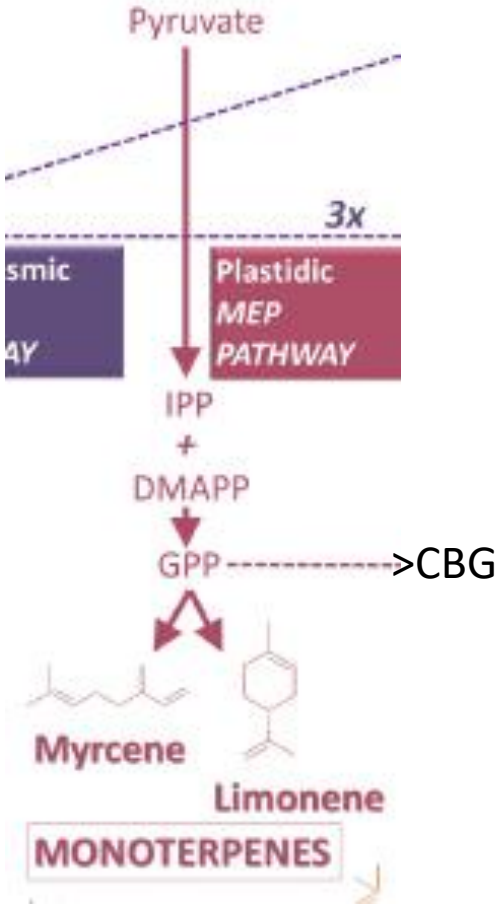
Observed a statistically significant 21% increase in cannabinoid content post-treatment ($P < 0.05$)

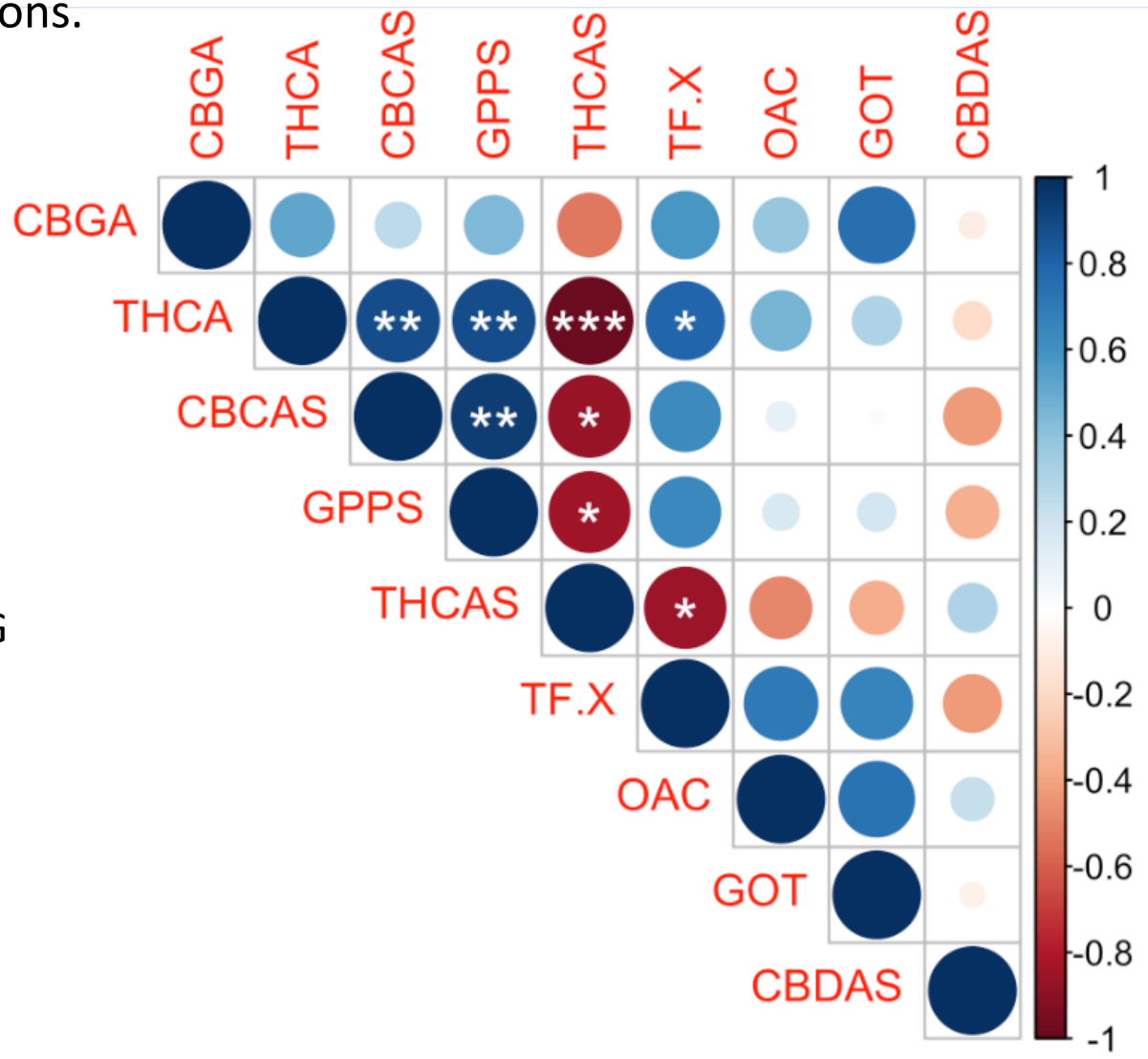


Pearson's Product-Moment Correlation Matrix evaluating the positive (●) and negative (●) relationships between cannabinoids and biosynthetic pathway enzymes in medical marijuana. Shade and size of circles denote extent of correlation. Stars indicate significance (5, 1, and 0.1%) evaluated using ANOVA.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

<u>What have we learned?</u>

1. 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 breeders-looking for increased cannabinoids as well as monoterpenes.

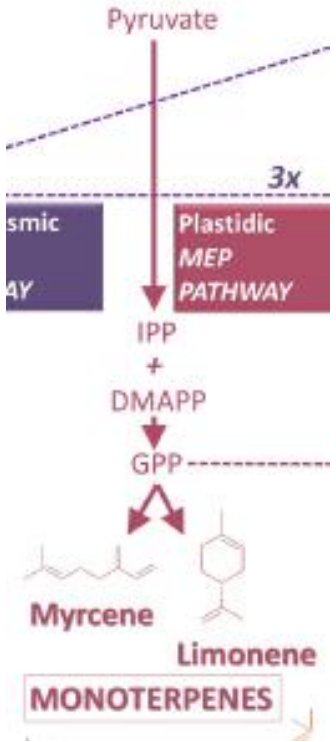


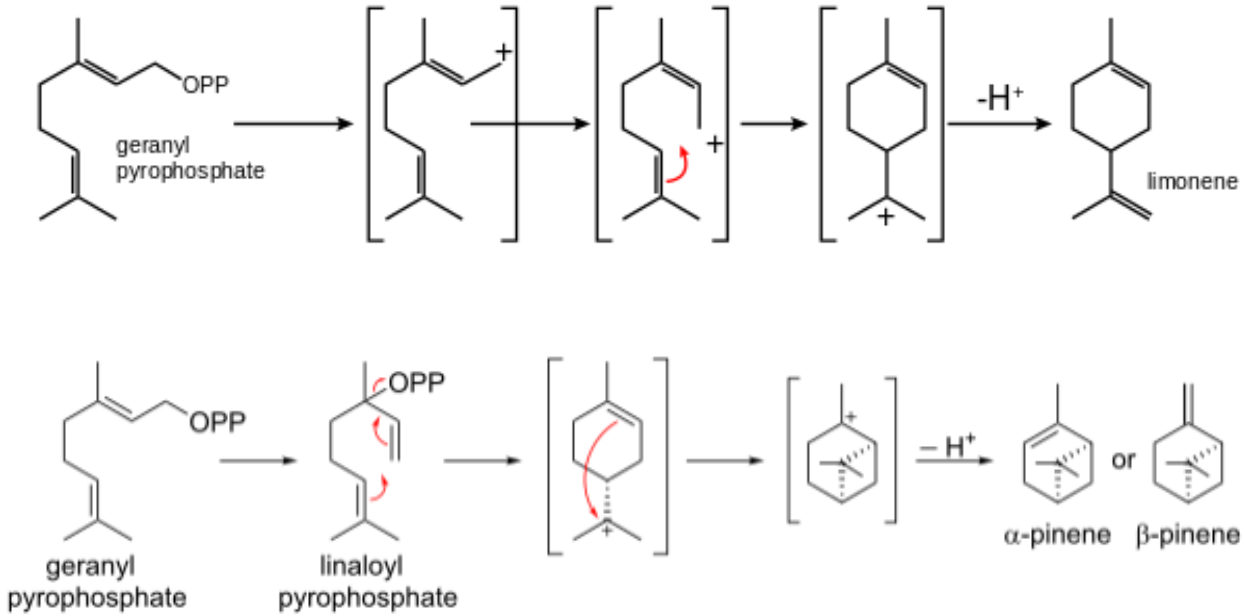


frontiers in Plant Science

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





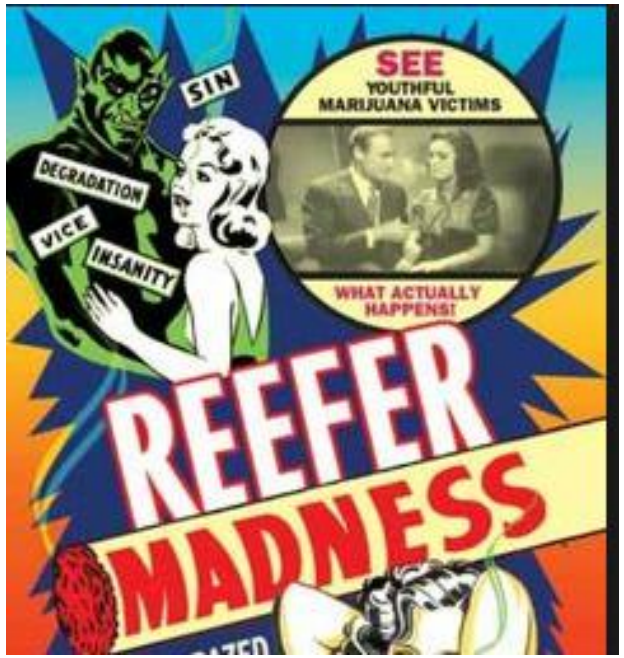
Where, for goodness sake, did the standard of 0.3% THC by weight come from?

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% Δ^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%.