Project WECANN: Investigating the diversity of wild *Cannabis*

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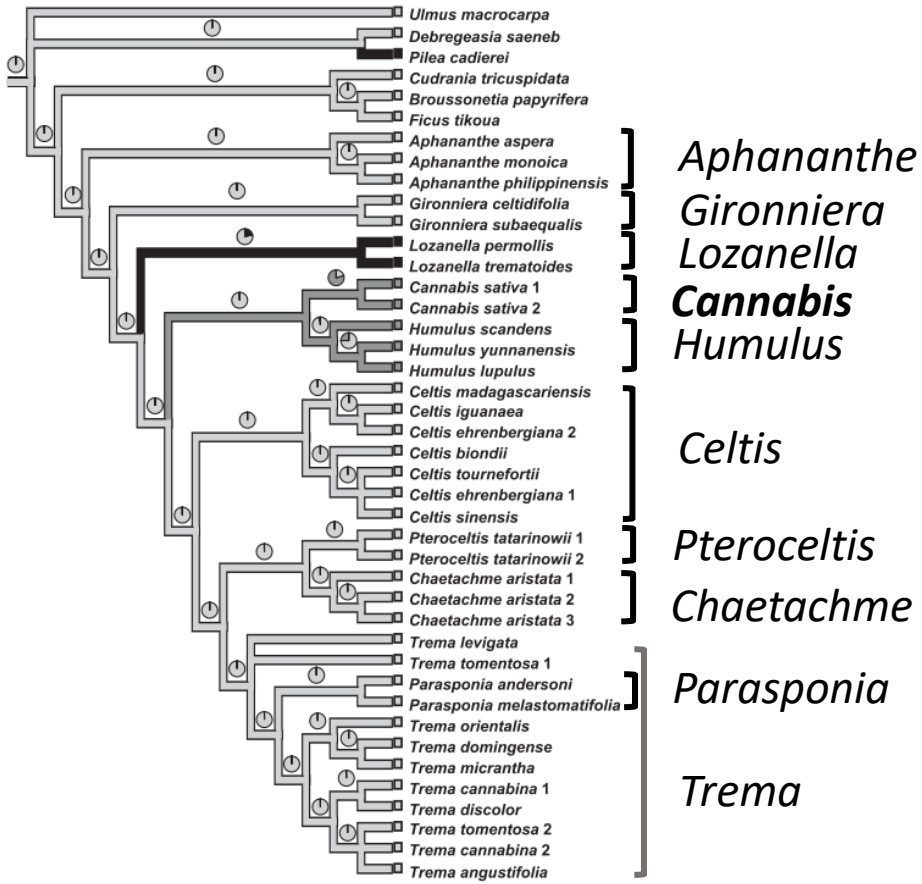
institut botànic
Centre mixt
CSIC

UNIVERSITAT DE BARCELONA

Family Cannabaceae



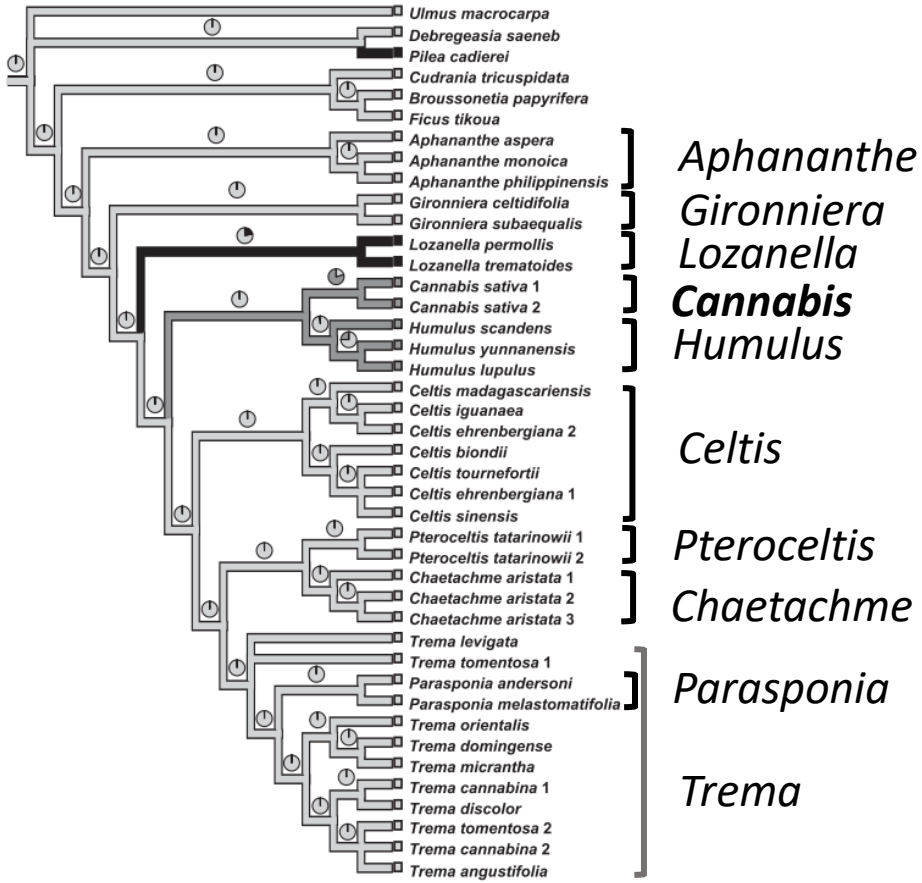
Jang et al. 2013

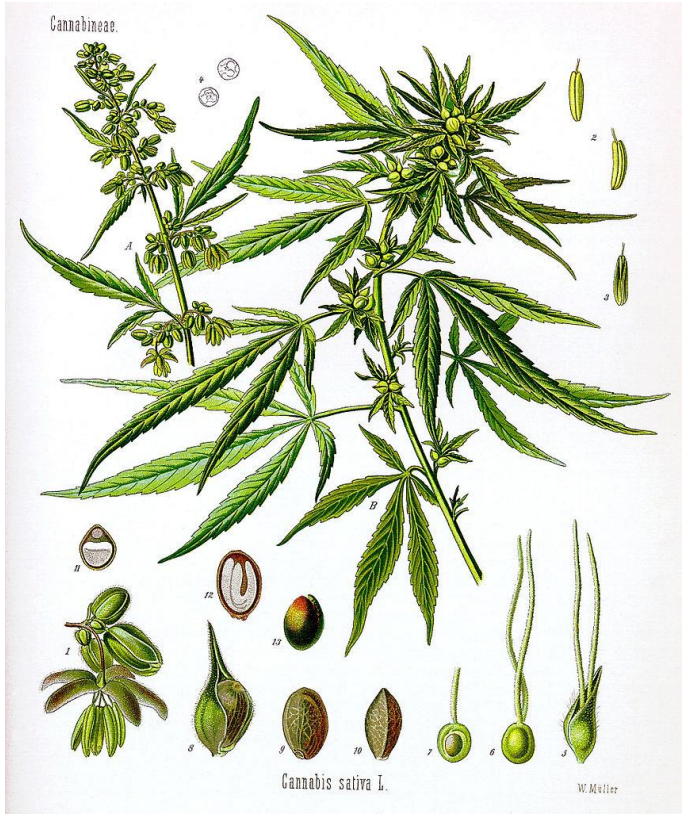
Humulus lupulus

Celtis australis

Trema orientalis

Family Cannabaceae





Jang et al. 2013

Family Cannabaceae

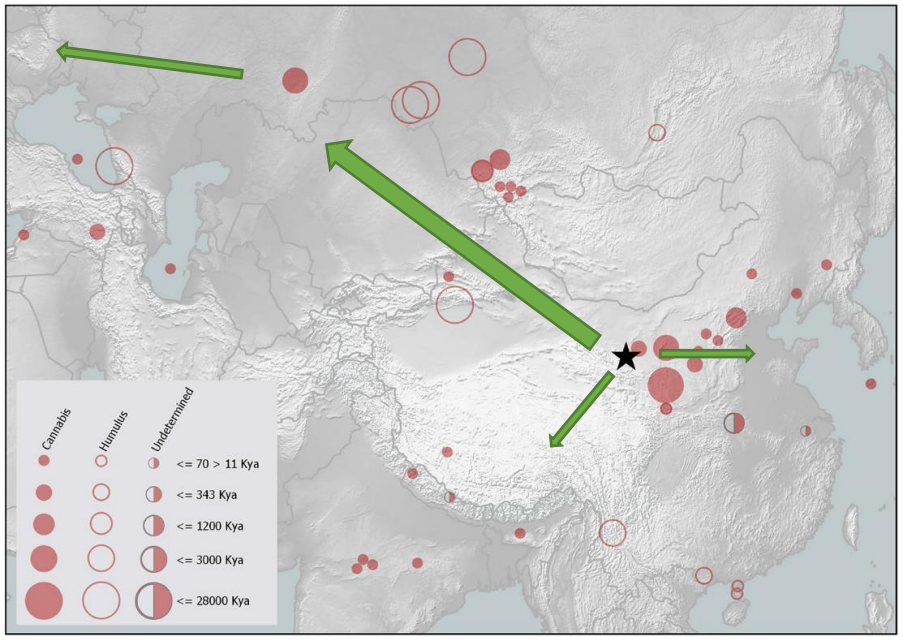
- Steppe habitat
- Phenotypic variability

Family Cannabaceae

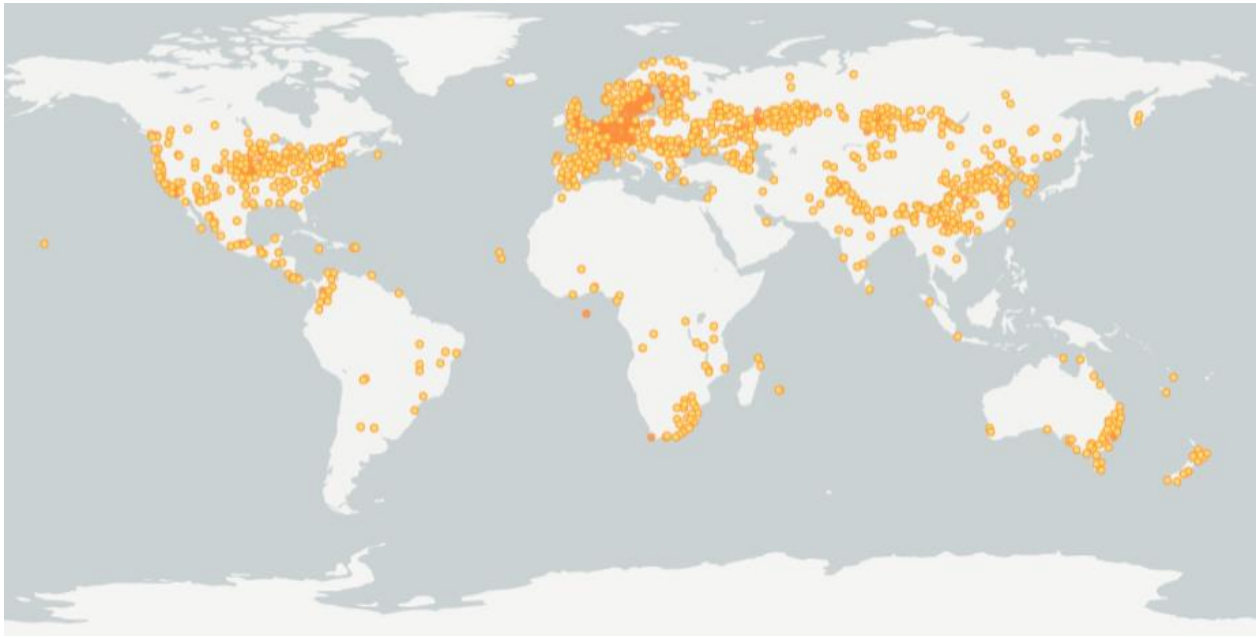
- Dioecious plants (monoecious)
- 2n = 20 (Males XY + 18 / Females XX + 18 / Hermaphrodites XX + 18)

Where *Cannabis* comes from?

- Origin – NE Tibetan Plateau ~27.8 mya
- Oldest pollen reference ~19.6 mya (China) – towards W (Volga ~1.5 mya) – finally Europe (Bulgaria ~ 6 mya) and to the E (China 1.2 mya) – later to India (32.6 kya)



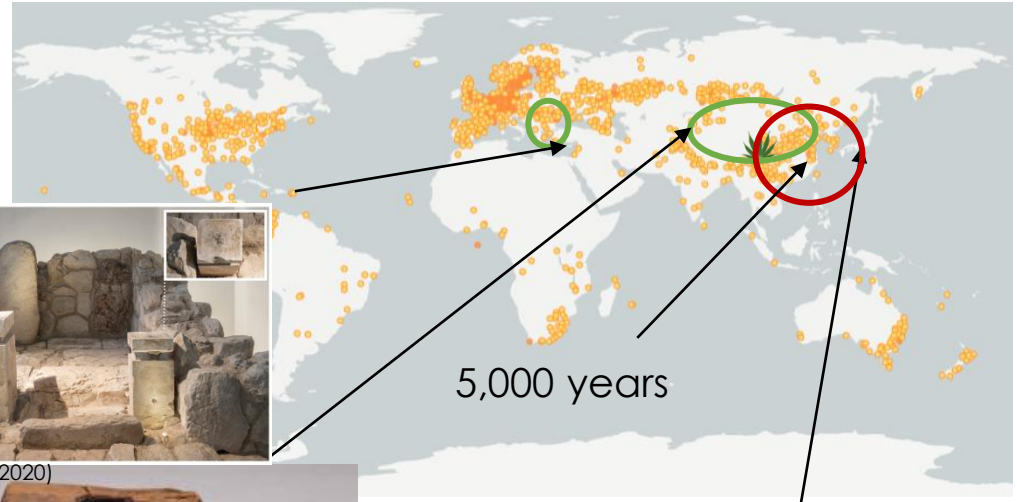
McPartland et al. 2019



Source: GBIF

Where *Cannabis* comes from?

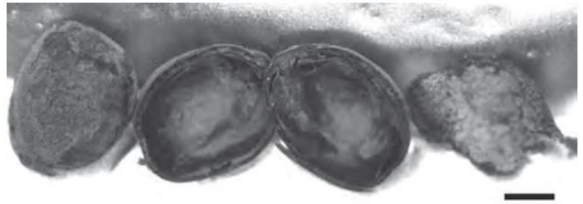
- Domestication area



(Arie et al., 2020)

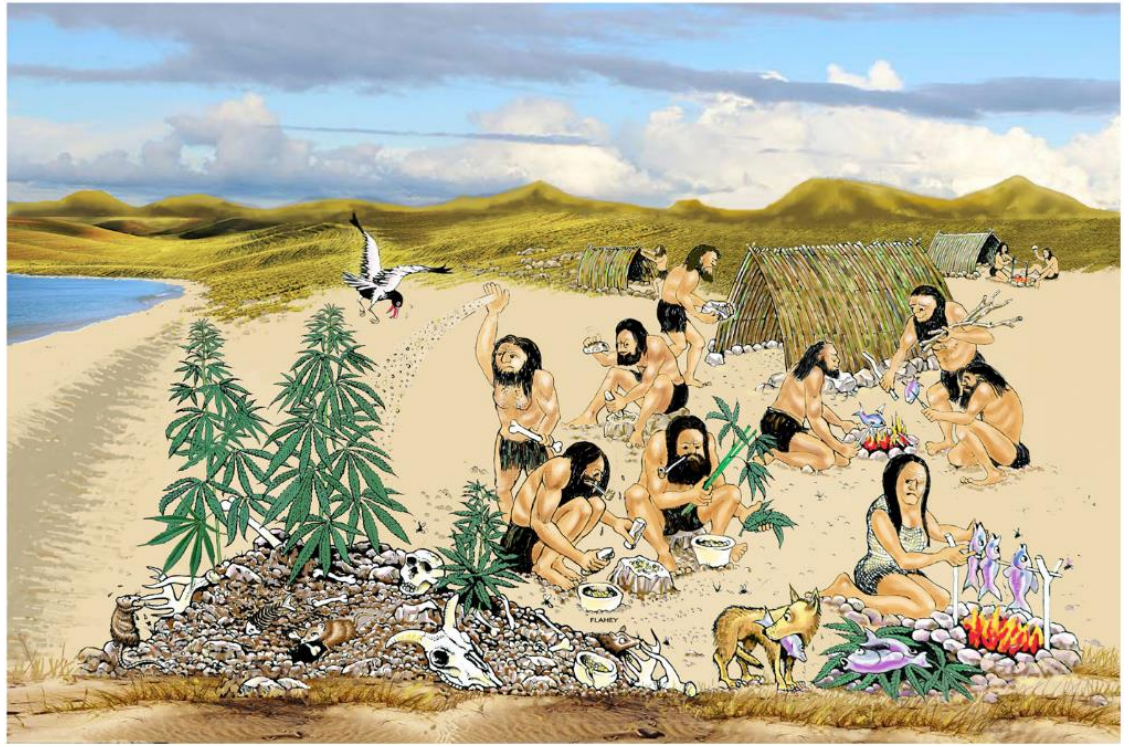
(Ren et al., 2019)

2,700 years



(Kudo et al., 2009)

10,000 years



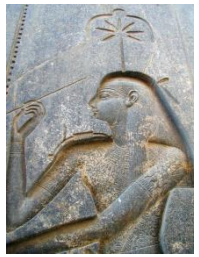
(Small, 2015)

Cannabis – traditional uses

- Psychoactive and religious use



- Medicinal use

ZEND - AVESTA
ZEN-DASCHTA
DU VENDIDAD


- Alimentary use



- Fibre use

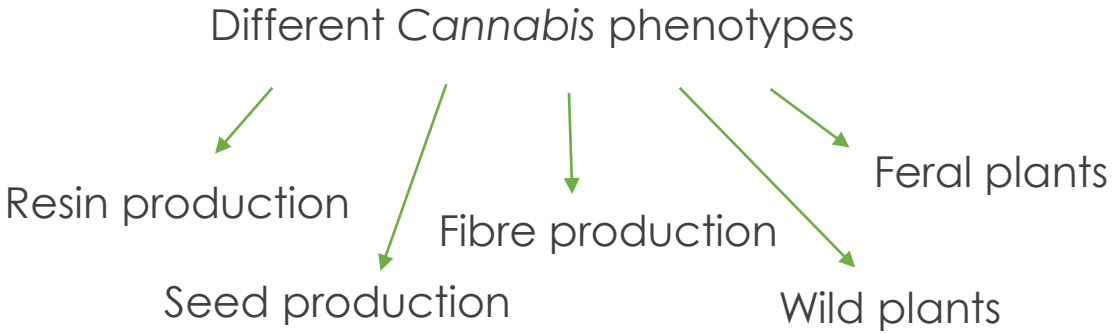


- Other uses



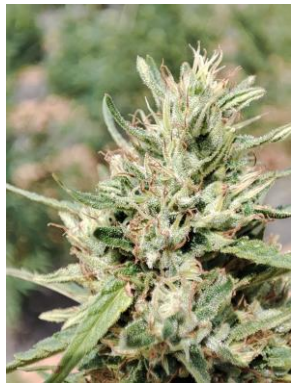
...

Cannabis – origin and taxonomic questions



Cannabis – origin and taxonomic questions

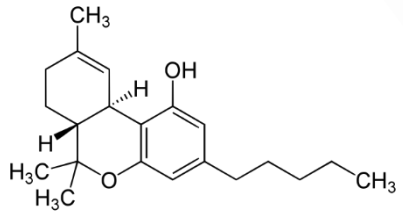
Different *Cannabis* phenotypes



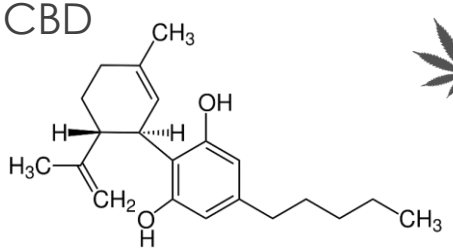
Cannabis – Chemical composition

- More than 150 cannabinoids and hundreds of other compounds

THC



CBD



Cannabis – Ch

- More than 150 cannabinoids and hundreds of other compounds
  - Anti-inflammatory
  - Antimicrobial
  - Analgesic
  - Neuroprotective
  - Antiarthritic
  - Antispasmodic
  - .....

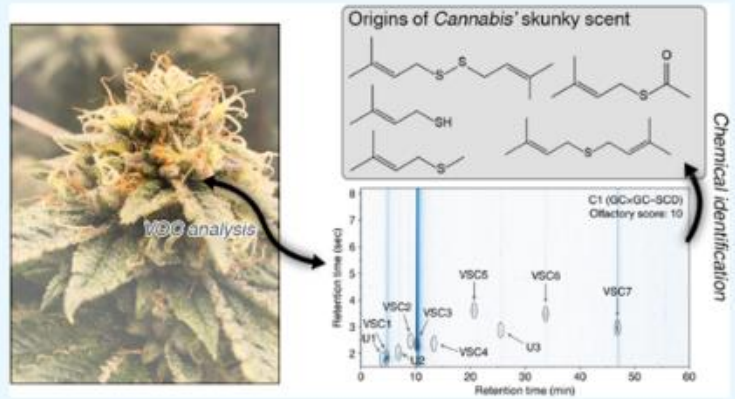
Medicinal applications

Identification of a New Family of Prenylated Volatile Sulfur Compounds in *Cannabis* Revealed by Comprehensive Two-Dimensional Gas Chromatography

Iain W. H. Oswald,* Marcos A. Ojeda, Ryan J. Pobanz, Kevin A. Koby, Anthony J. Buchanan, Josh Del Rosso, Mario A. Guzman, and Thomas J. Martin

Cite This: https://doi.org/10.1021/acsomega.1c04196 Read Online

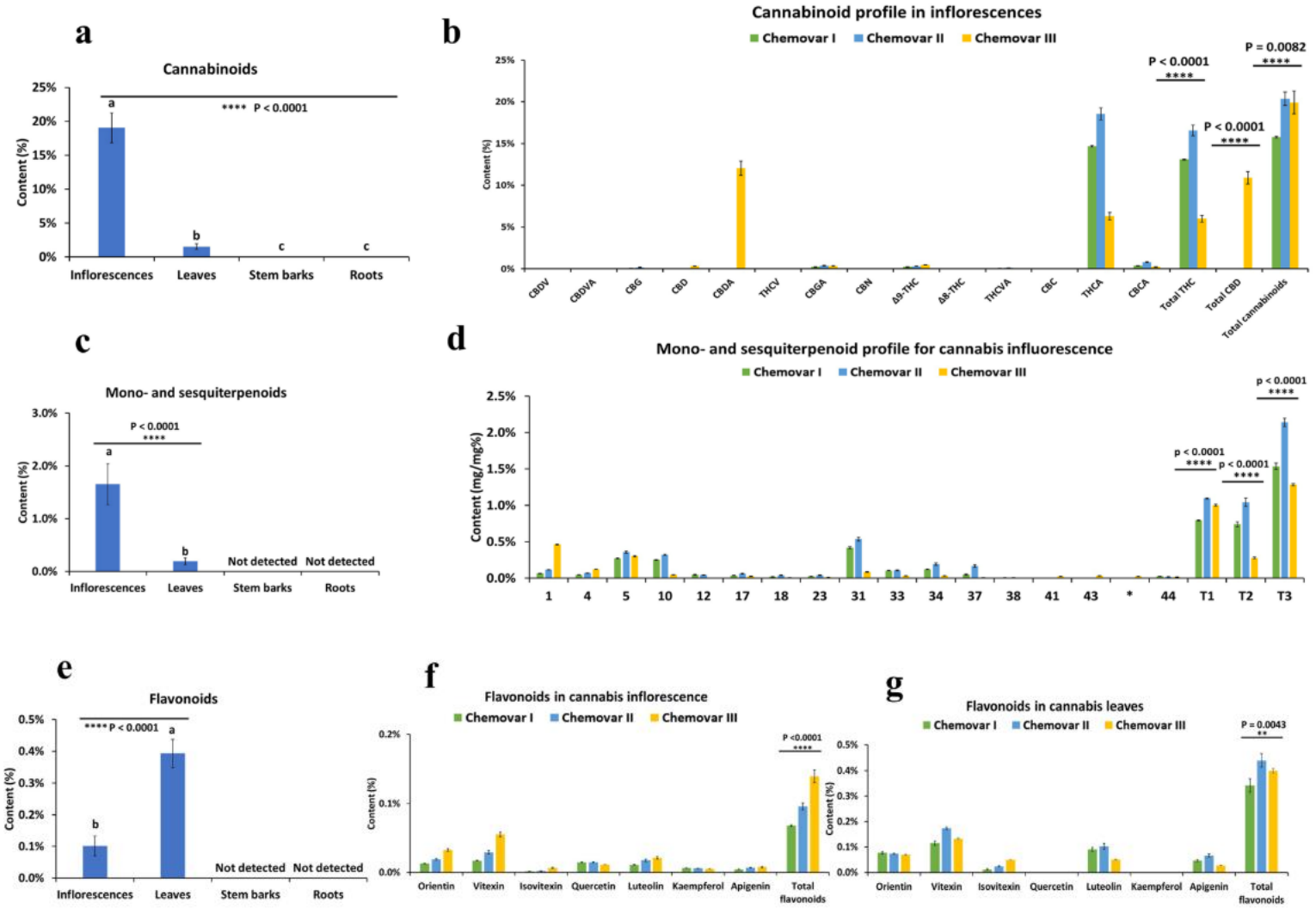
ABSTRACT: *Cannabis sativa* L. produces over 200 known secondary metabolites that contribute to its distinctive aroma. Studies on compounds traditionally associated with the scent of this plant have focused on those within the terpenoid class. These isoprene-derived compounds are ubiquitous in nature and are the major source of many plant odors. Nonetheless, there is little evidence that they provide the characteristic "skunk-like" aroma of *cannabis*. To uncover the chemical origins of this scent, we measured the aromatic properties of *cannabis* flowers and concentrated extracts using comprehensive two-dimensional gas chromatography equipped with time-of-flight mass spectrometry, flame ionization detection, and sulfur chemiluminescence. We discovered a new family of volatile sulfur compounds (VSCs) containing the prenyl (3-methylbut-2-en-1-yl) functional group that is responsible for this scent. In particular, the compound 3-methyl-2-butene-1-thiol was identified as the primary odorant. We then conducted an indoor greenhouse experiment to monitor the evolution of these compounds during the plant's lifecycle and throughout the curing process. We found that the concentrations of these compounds increase substantially during the last weeks of the flowering stage, reach a maximum during curing, and then drop after just one week of storage. These results shed light on the chemical origins of the characteristic aroma of *cannabis* and how volatile sulfur compound production evolves during plant growth. Furthermore, the chemical similarity between this new family of VSCs and those found in garlic (*allium sativum*) suggests an opportunity to also investigate their potential health benefits.



Jin et al 2020

Cannabis – Chemical composition

- Genetics
- Position within a plant
- Plant part



Jin et al 2020

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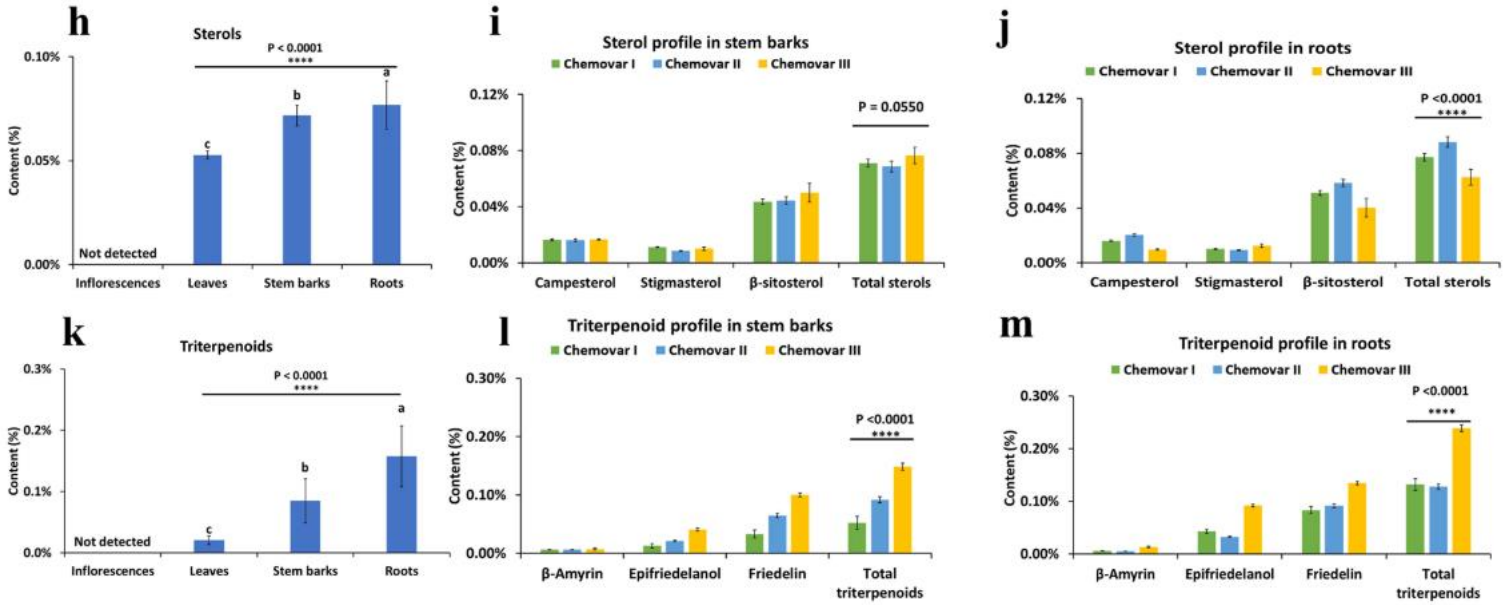
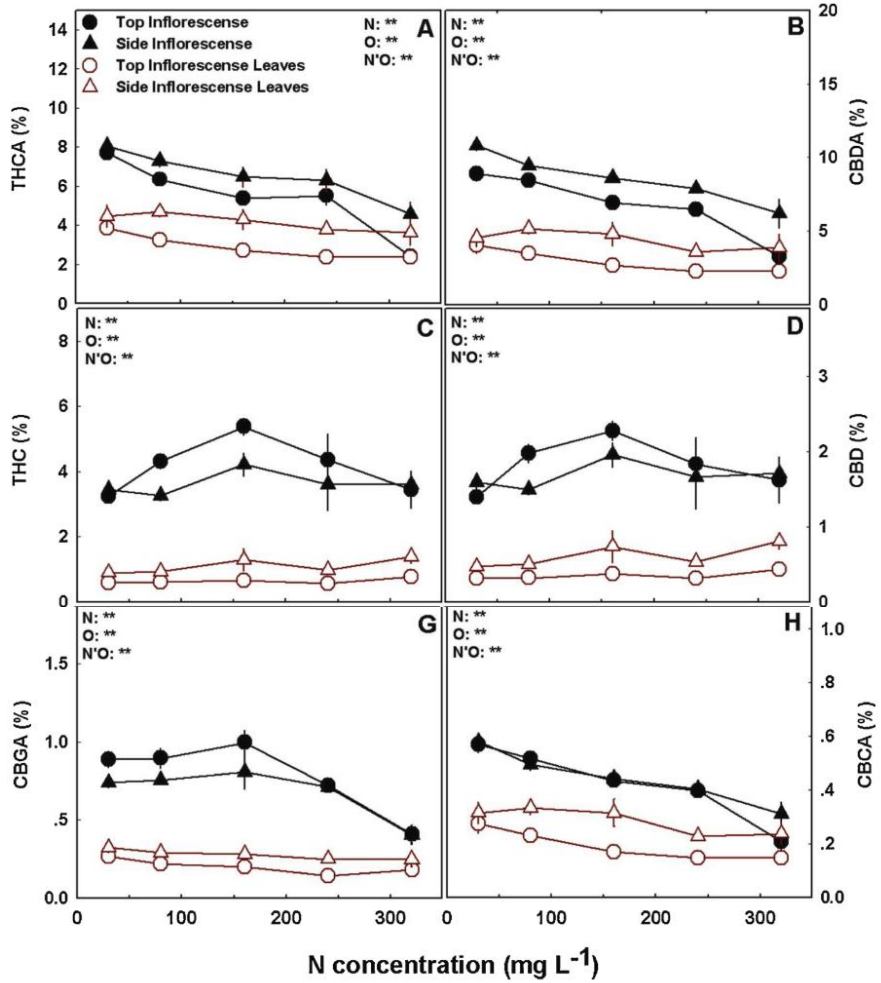


Jin et al 2020

Cannabis – Chemical composition

- Genetics
- Position within a plant
- Plant part
- Growing conditions
  - Soil
  - Light intensity & specter
  - Nutrition
  - Irrigation
  - pH



Saloner & Bernstein, 2021

Cannabis – origin and taxonomic questions

- How many species, subspecies, varieties are there?

C. sativa* subsp. *indica* var. *himalayensis

?

C. sativa* subsp. *indica* var. *asperrima

Cannabis ruderalis

Cannabis sativa* var. *indica

?

Cannabis sativa* subsp. *ruderalis

Cannabis sativa* subsp. *sativa

Cannabis sativa* var. *ruderalis

***Cannabis sativa* L.**

Cannabis indica

Cannabis sativa* subsp. *indica

Cannabis sativa* var. *sativa

?

?

Cannabis indica* subsp. *indica

Cannabis indica* subsp. *sativa

Cannabis spontanea

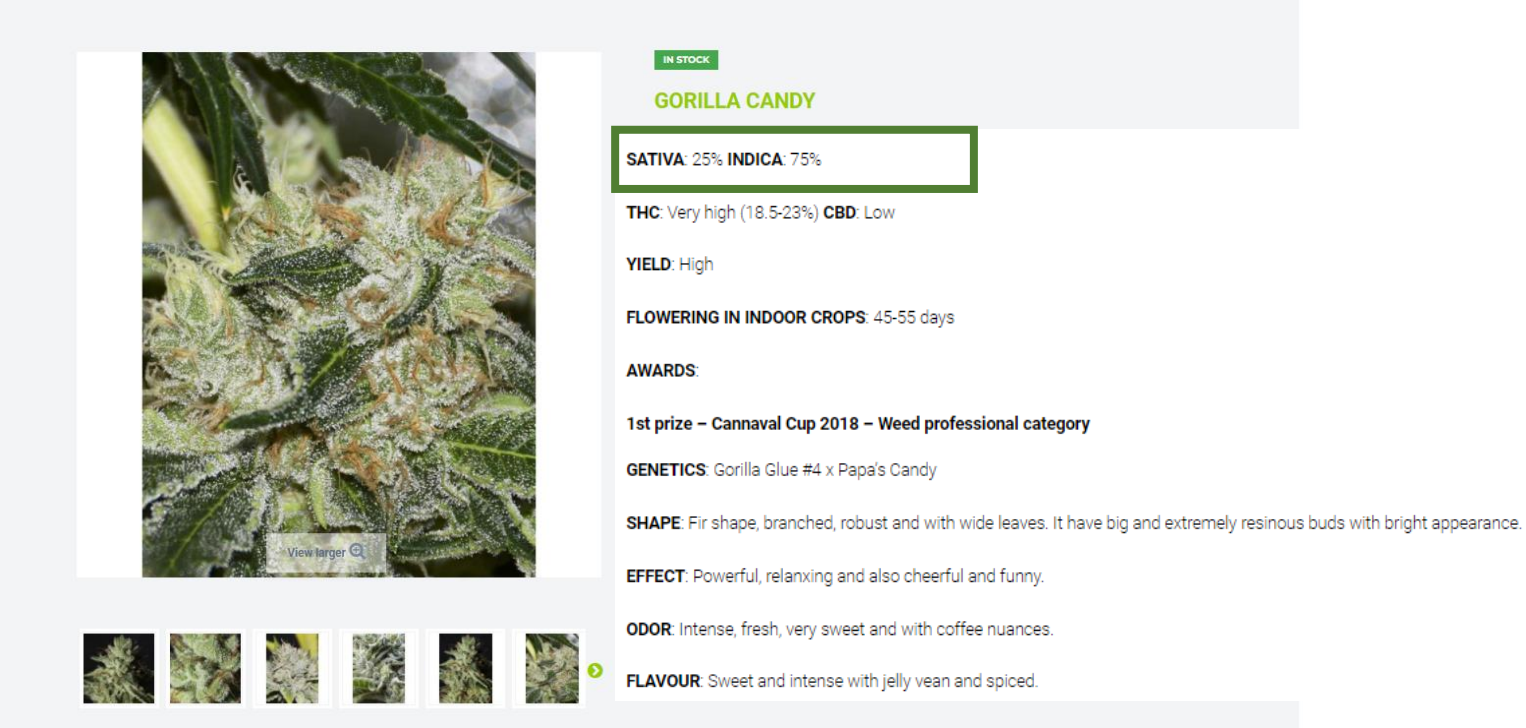
C. sativa* subsp. *indica* var. *indica

C. sativa* subsp. *indica* var. *afghanica

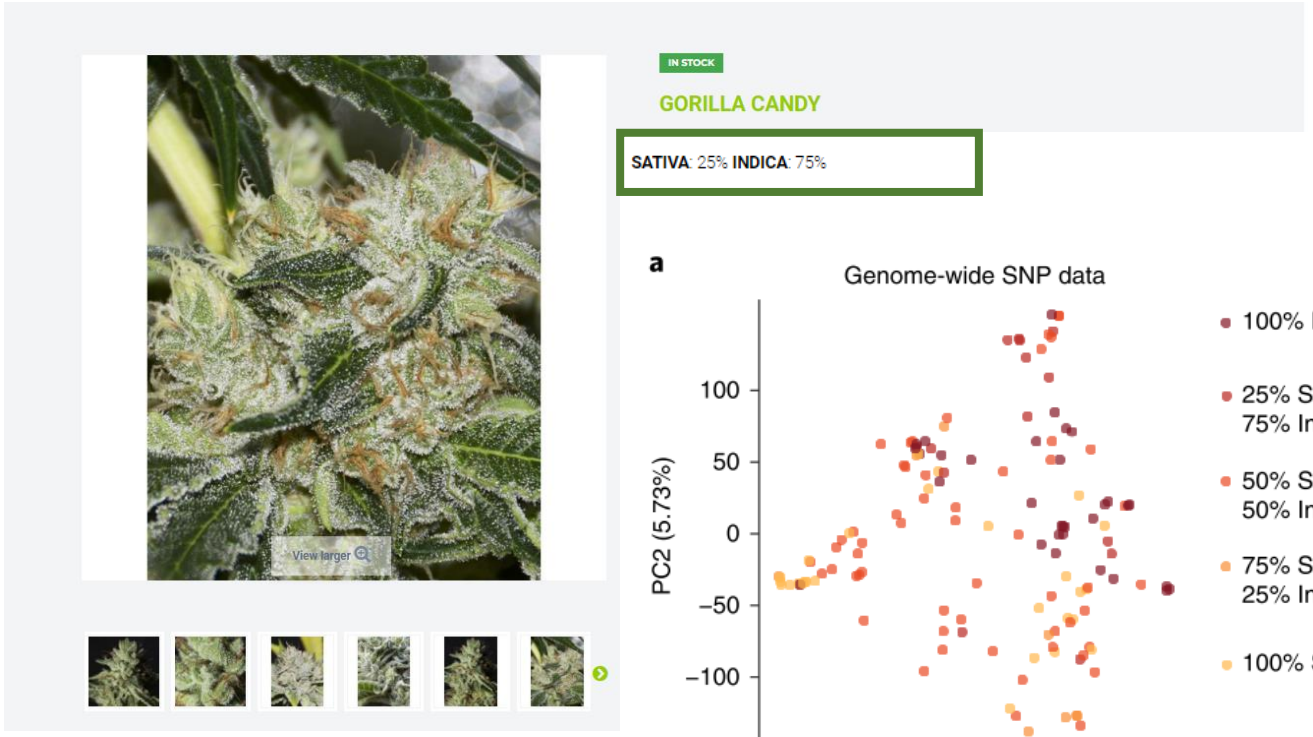
C. sativa subsp. indica sect. spontanea var. spontanea

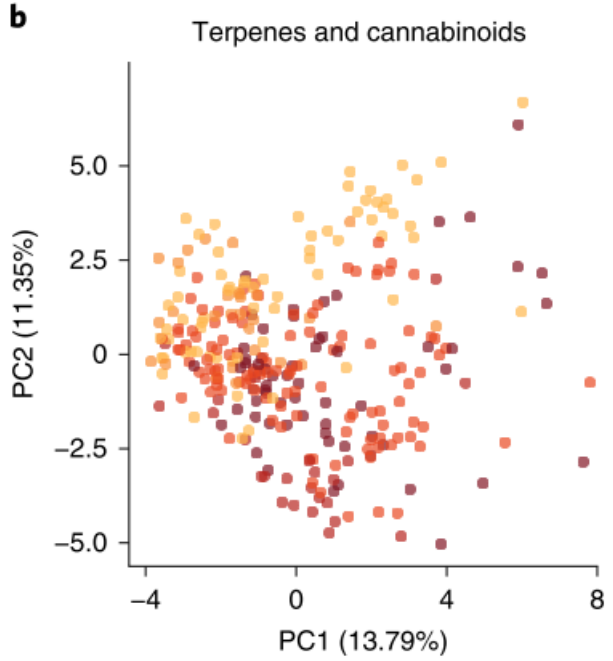
C. ruderalis *C. indica* *C. sativa*

Cannabis – origin and taxonomic questions



Cannabis – origin and taxonomic questions





Watts et al 2021

Cannabis – origin and taxonomic questions

Ancestral populations

Natural dispersion

First human utilisation

Cultivation

Initial selection for different uses - land races

Human dispersal within Asia and Secondary selection

Human dispersal across the world

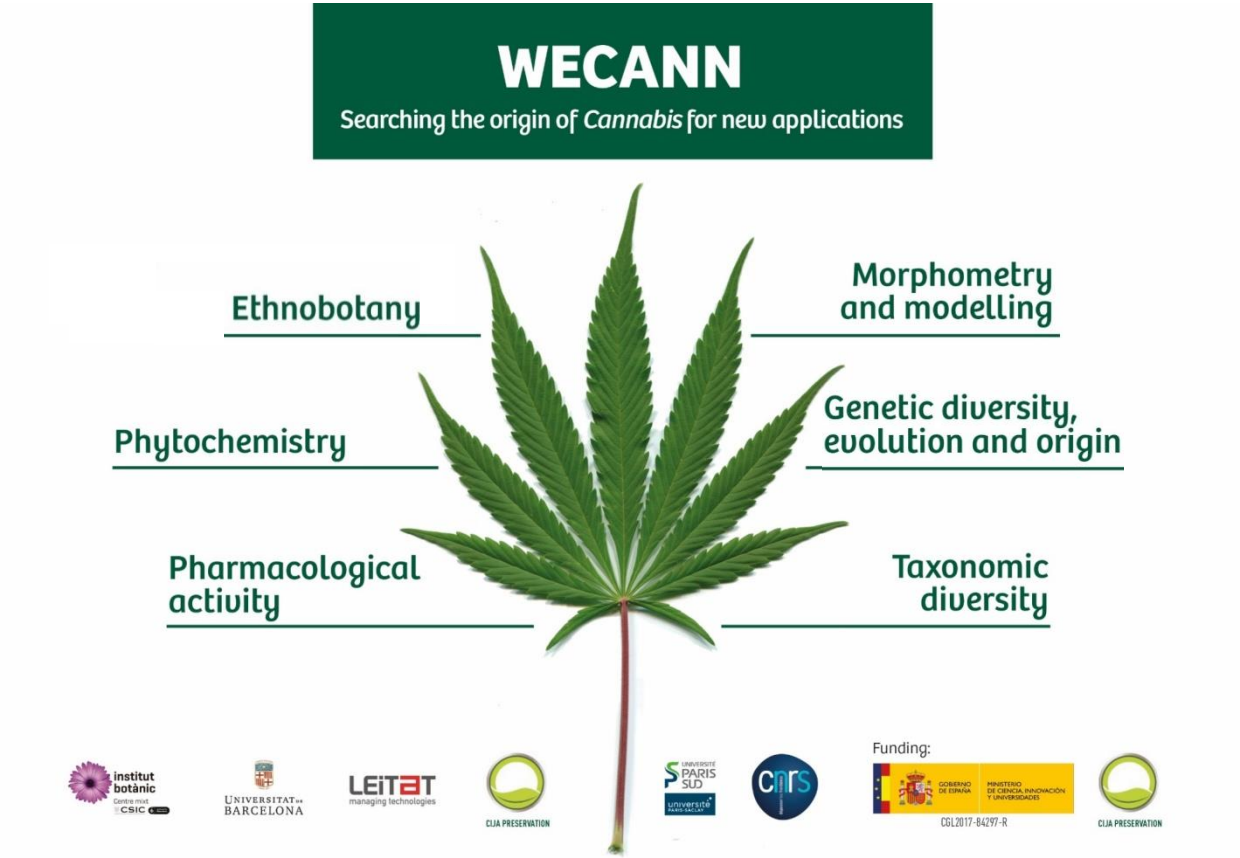
Modern cultivars

Cultivar escapes

Human dispersal across the world

C. ruderalis *C. indica* *C. sativa*

Project objectives



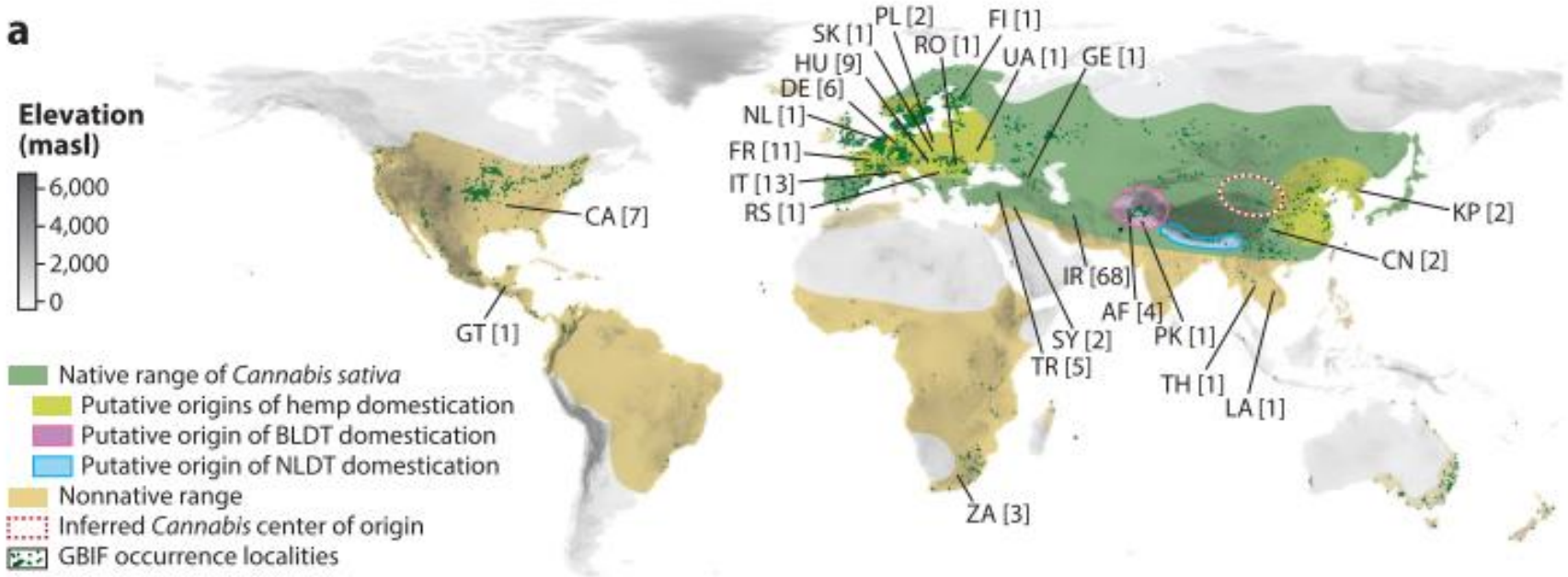
Taxonomic and genetic diversity of *Cannabis*

ANNUAL REVIEWS

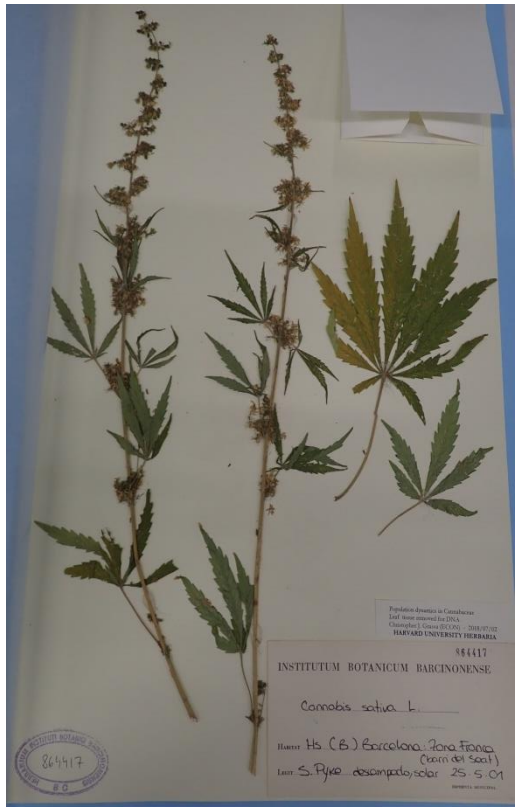
Annual Review of Plant Biology

The Genomics of *Cannabis* and Its Close Relatives

I. Kovalchuk,[1] M. Pellino,[2] P. Rigault,[3,4]
R. van Velzen,[5,6] J. Ebersbach,[7] J. R. Ashnest,[2] M. Mau,[2]
M. E. Schranz,[5] J. Alcorn,[8] R. B. Laprairie,[8,9]
J. K. McKay,[10] C. Burbridge,[11] D. Schneider,[11]
D. Vergara,[12] N. C. Kane,[12] and T. F. Sharbel[2]



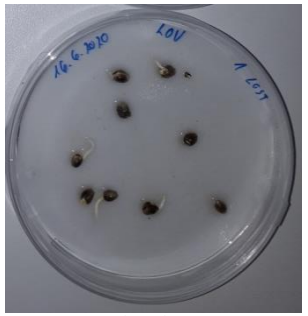
Taxonomic and genetic diversity of *Cannabis*



HybSeq approach with Angiosperms353 probe set (Johnson, Pokorny, Dodsworth et al 2018, Syst. Biol.)

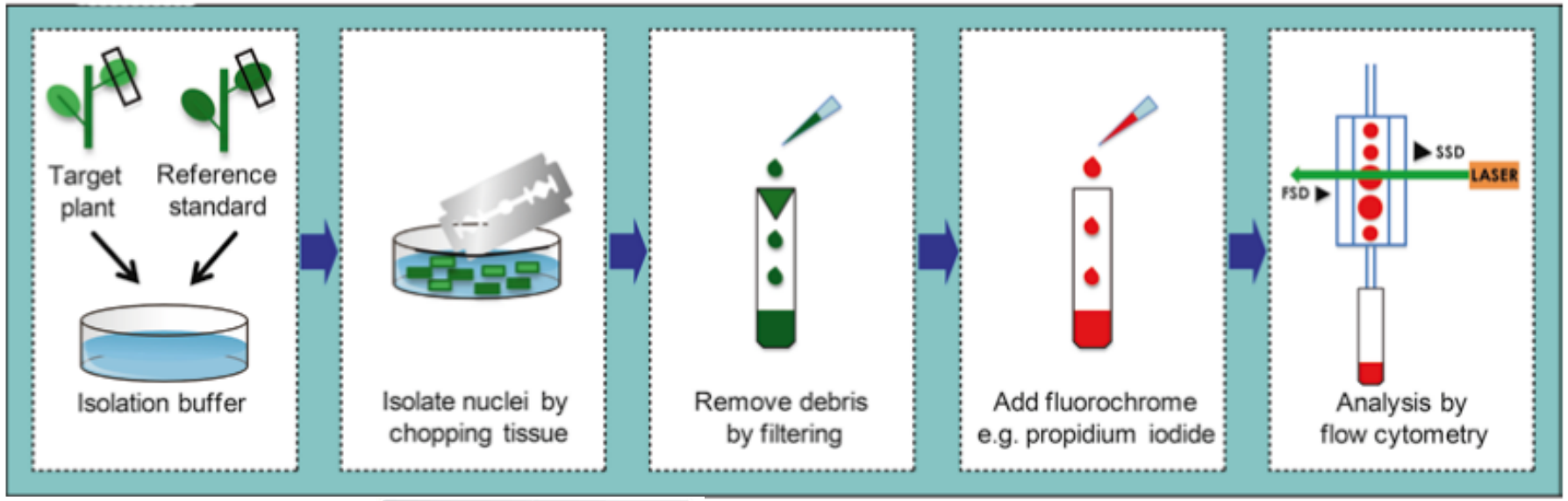
Taxonomic and genetic diversity of *Cannabis*

- Genome size – total amount of DNA within a nucleus of a cell

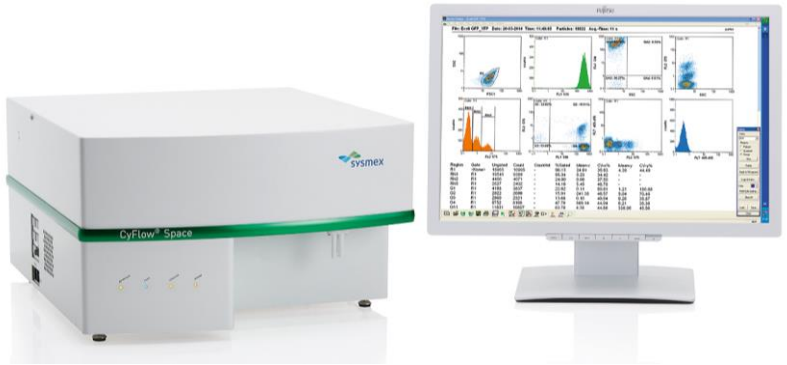


Seed germination

Plant cultivation



Pellicer & Leitch 2014



Taxonomic and genetic diversity of *Cannabis*

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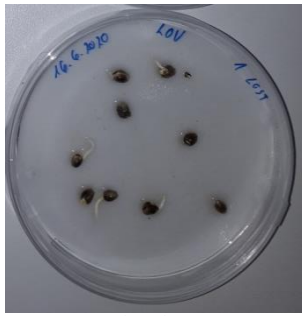

Seed germination

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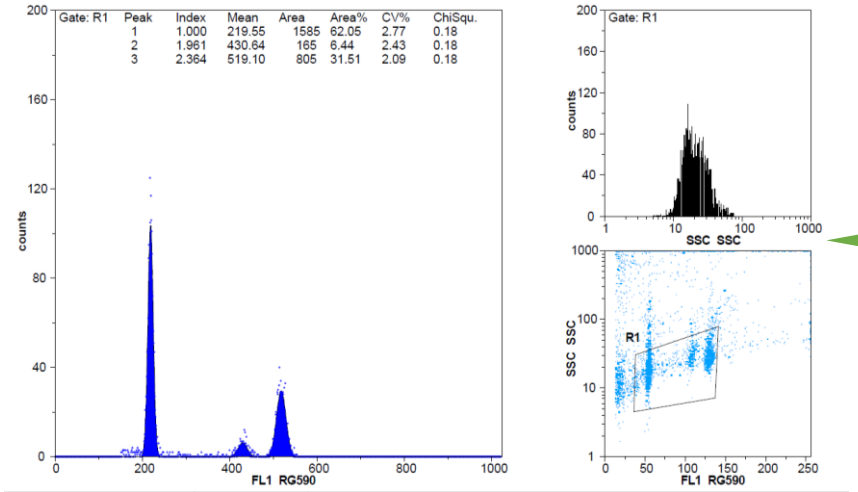
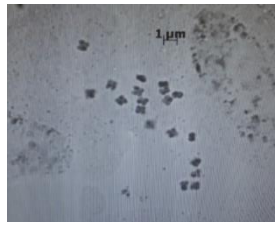
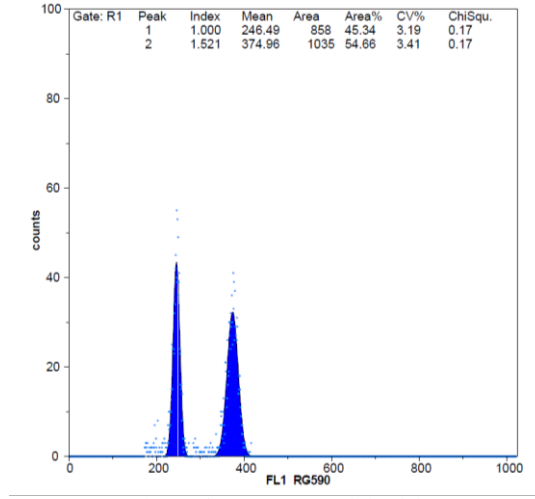
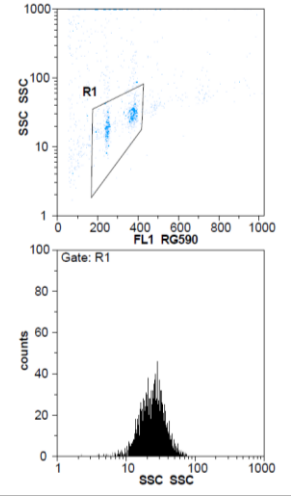


Assessment of genome size



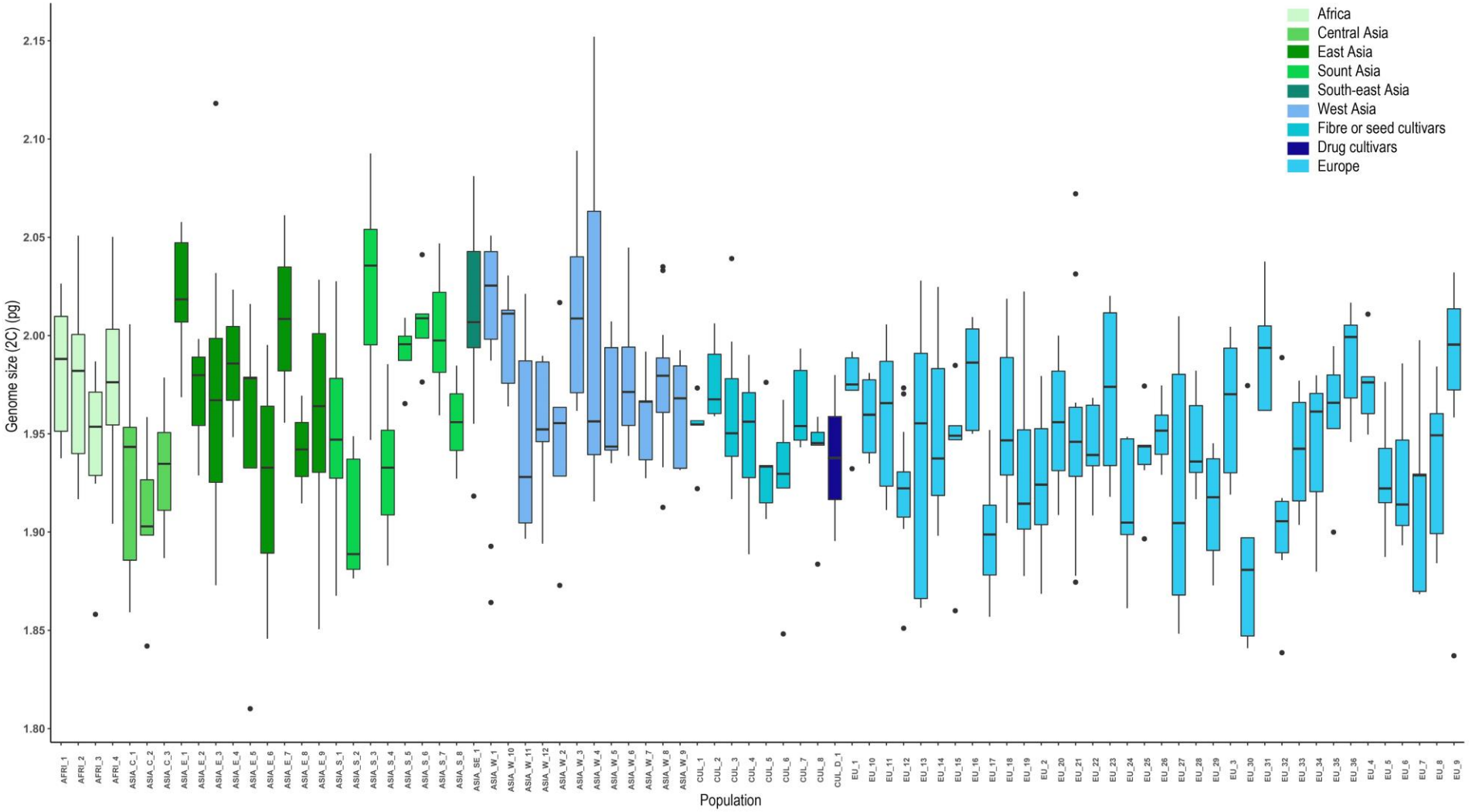
Chromosome counts





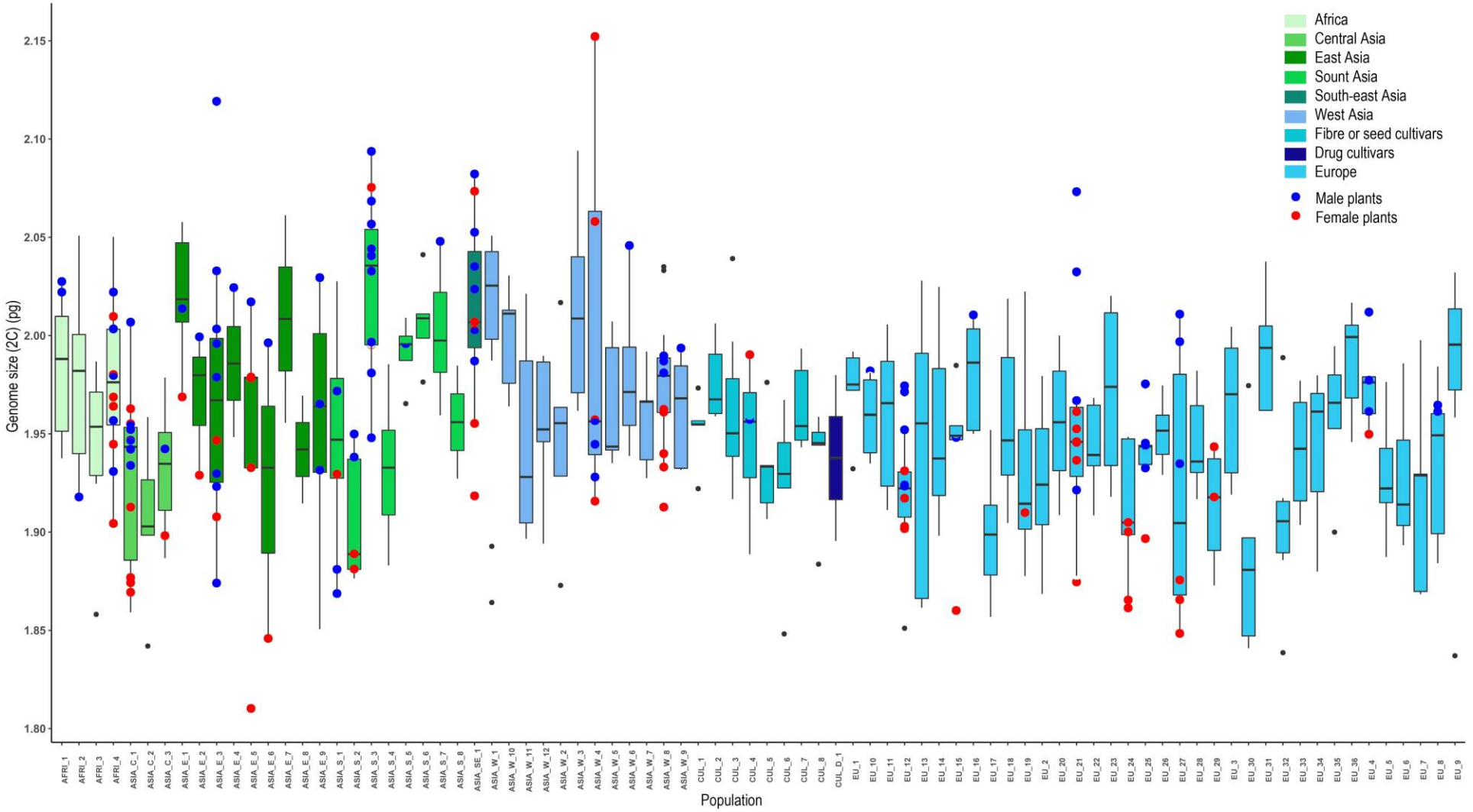
Comparison of two *Cannabis* individuals

Taxonomic and genetic diversity of *Cannabis*



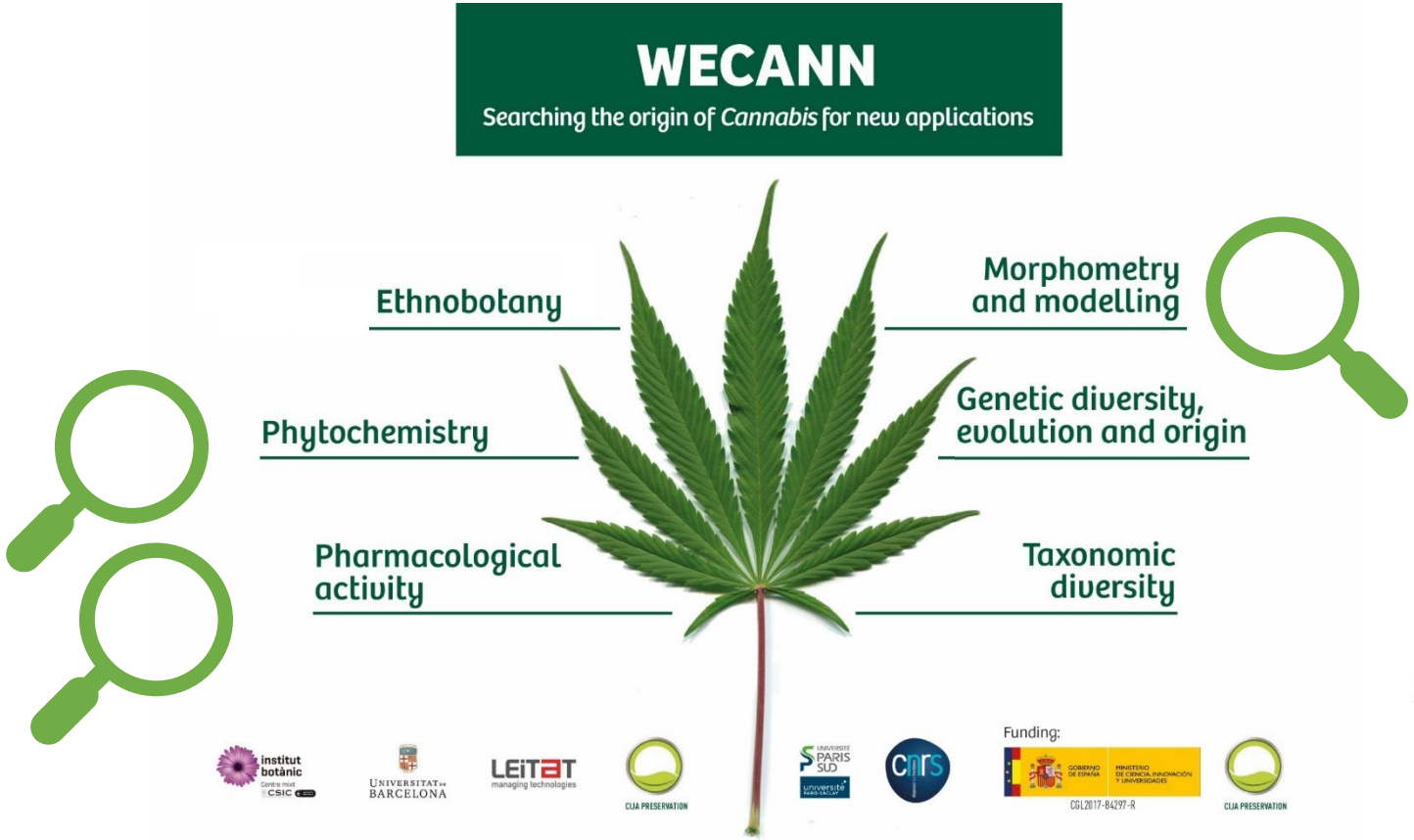
- Average GS 1.96pg (2C)
- 1.19-fold variation
- One triploid plant

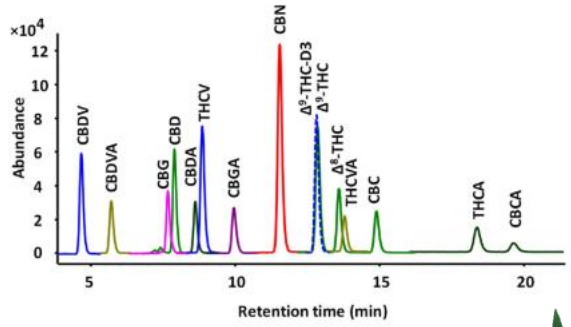
Taxonomic and genetic diversity of *Cannabis*



- Differences in sex ?
- Chemical compounds
- Repetitive elements

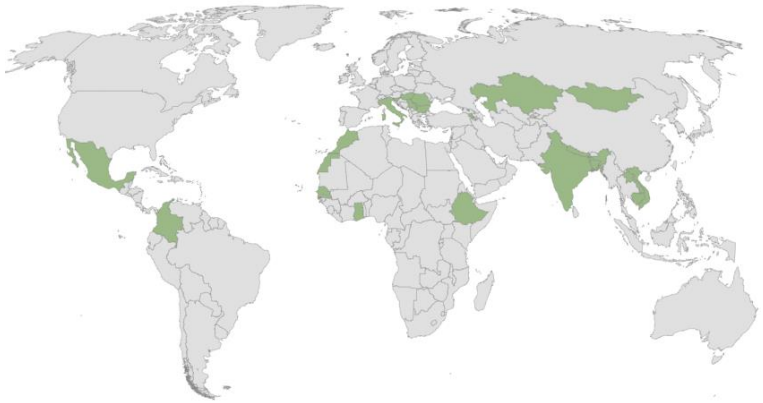
Cannabis – Morphometrics & Phytochemistry



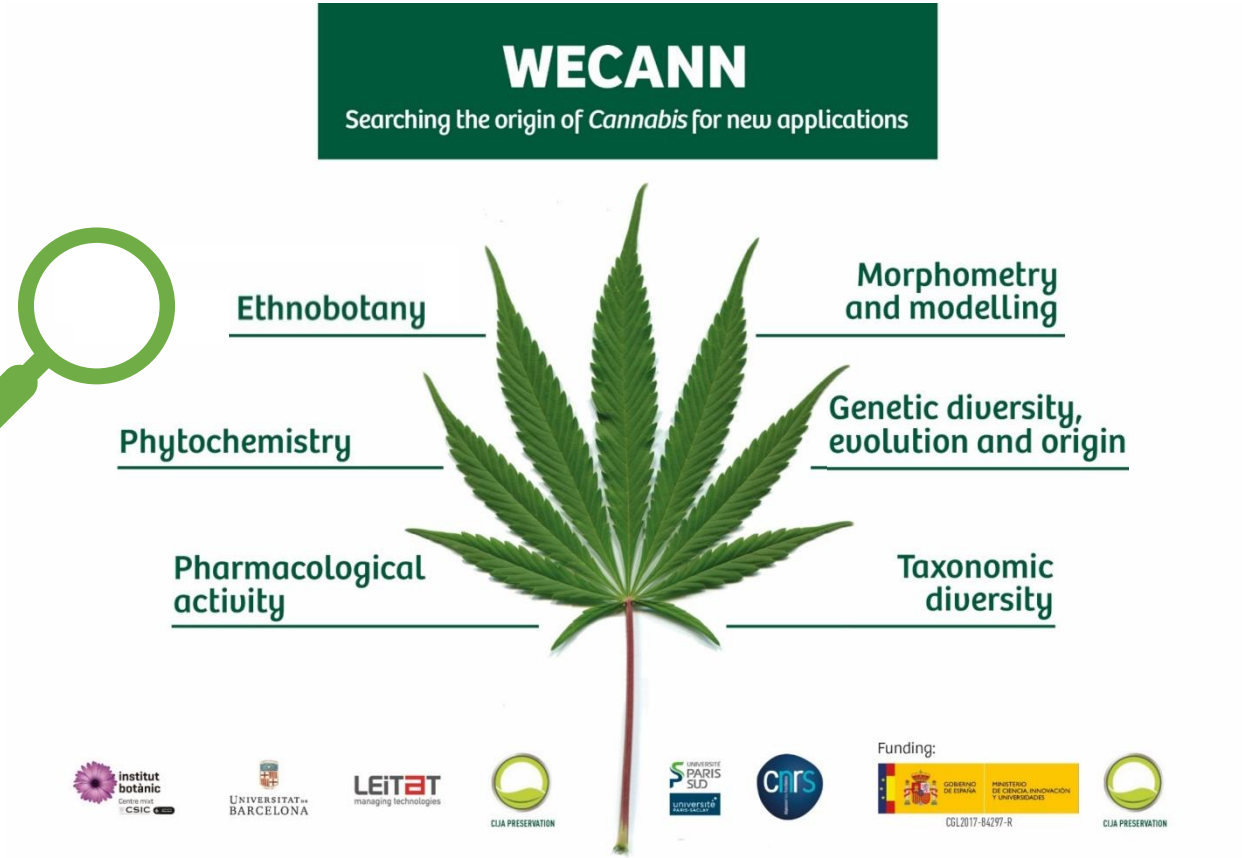


Species / subspecies differentiation?

Cannabis – Morphometrics & Phytochemistry



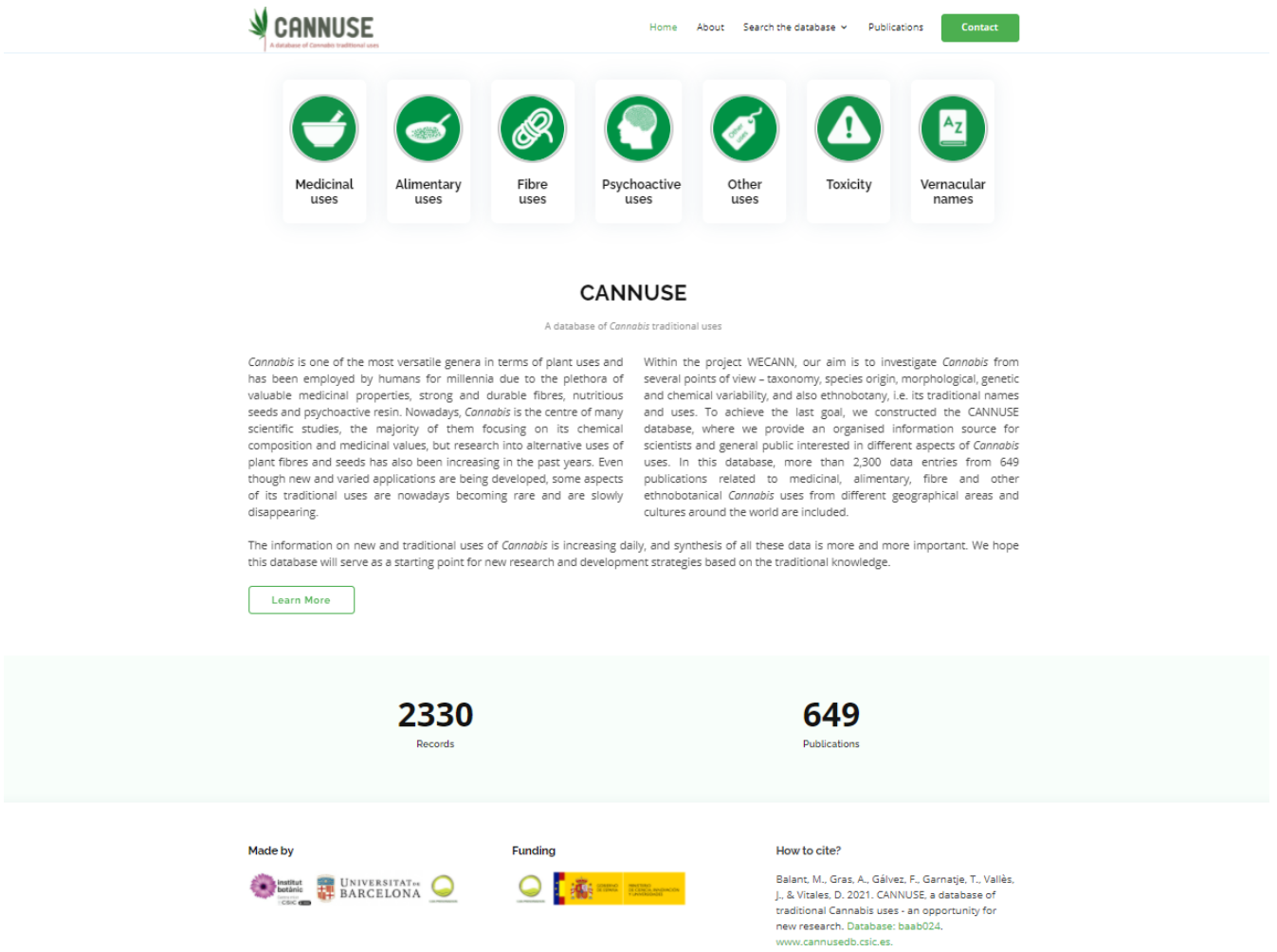
Project objectives



Cannabis – traditional uses

CANNUSE – A database of *Cannabis* traditional uses

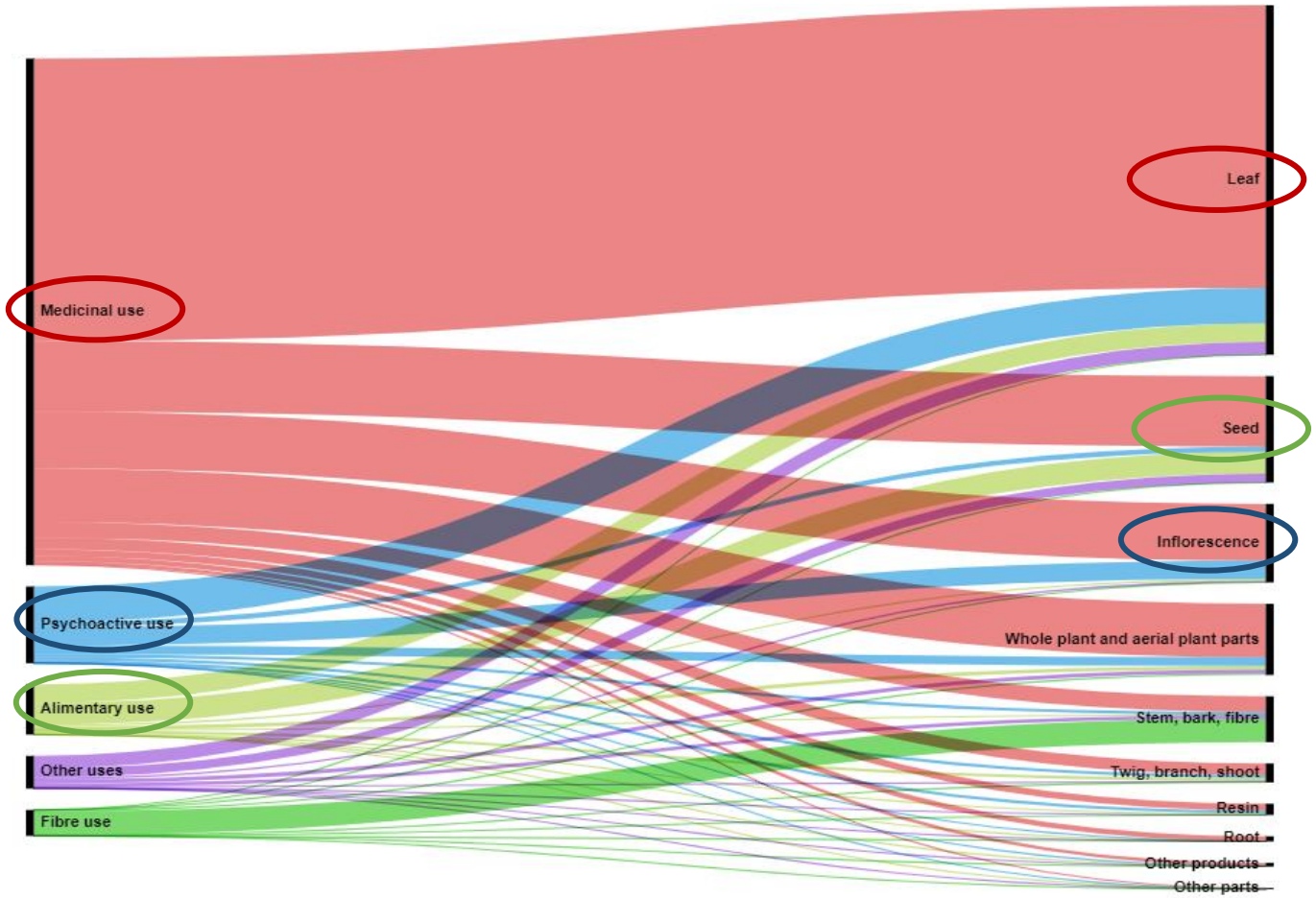
CANNUSE – A database of *Cannabis* traditional uses



https://cannusedb.csic.es/

Balant et al. CANNUSE, a database of traditional *Cannabis* uses—an opportunity for new research. Database (2021) Vol. 2021: article ID baab024; doi:10.1093/database/baab024

CANNUSE – A database of *Cannabis* traditional uses



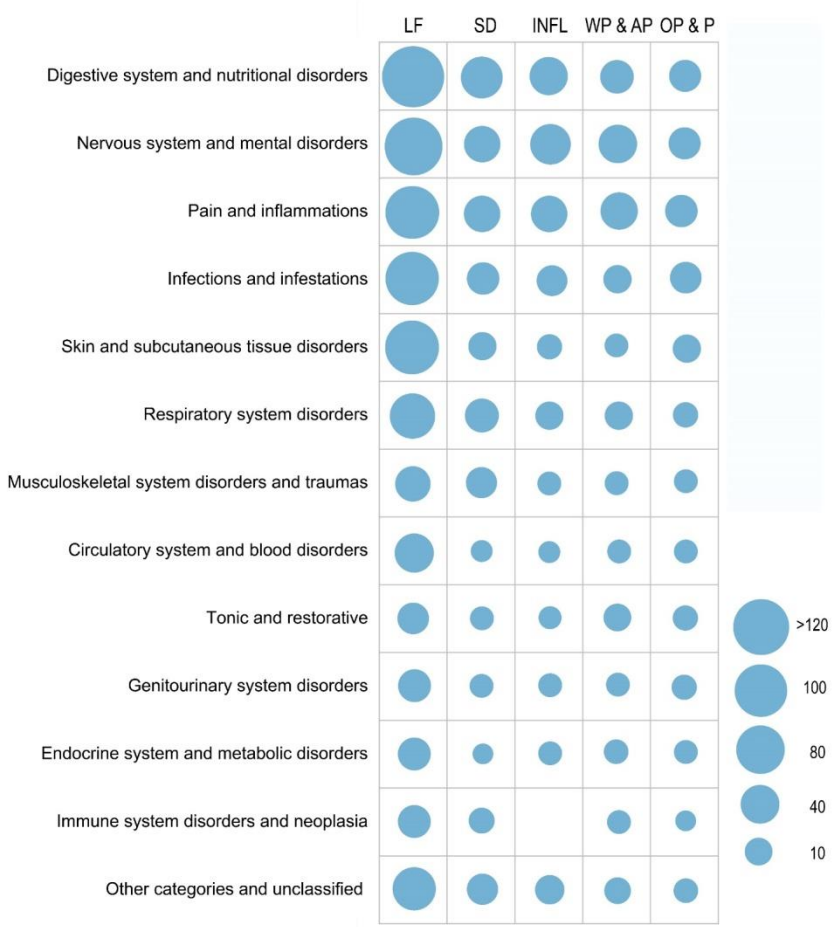
CANNUSE – A database of *Cannabis* traditional uses

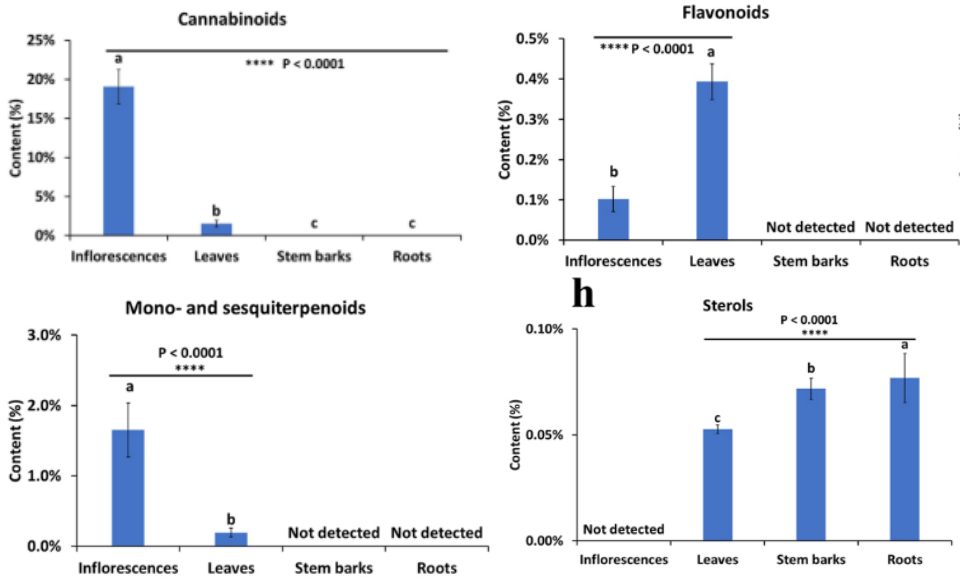
- Analysed 1167 data entries
  - 210 different ailments ⟶ 16 body system categories

Most commonly treated body systems and disorders:
- Digestive system and nutritional disorders
- Nervous system and mental disorders
- Pain and inflammations

Most common uses:
- Analgesic use
- Sedative use
- Antidiarrheal use
- Wound treatment
- Haemorrhoid treatment

CANNUSE – A database of *Cannabis* traditional uses





Jin et al. (2020)

Are all plant parts equally used for treatment of all body systems in traditional medicine?

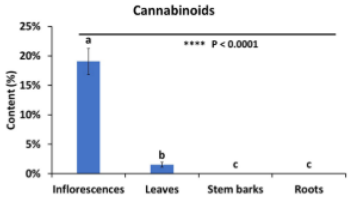
CANNUSE – A database of *Cannabis* traditional uses

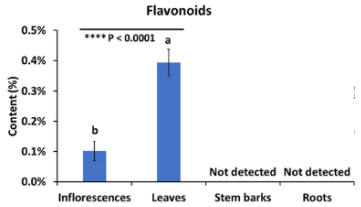
- Skin and subcutaneous tissue disorders
  - Wounds
  - Cuts
  - Fungal infections

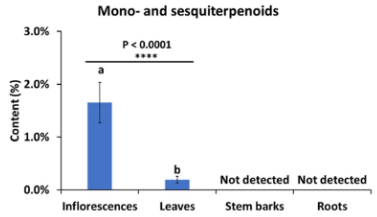
  ⟶ anti-inflammatory, anti-bacterial and anti-fungal properties

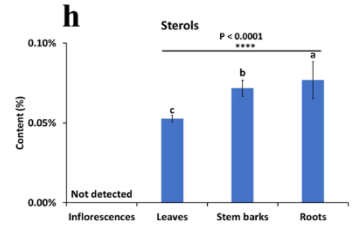
- Circulatory system and blood disorders
  - Haemorrhoid treatment

  ⟶ anti-inflammatory, analgesic properties









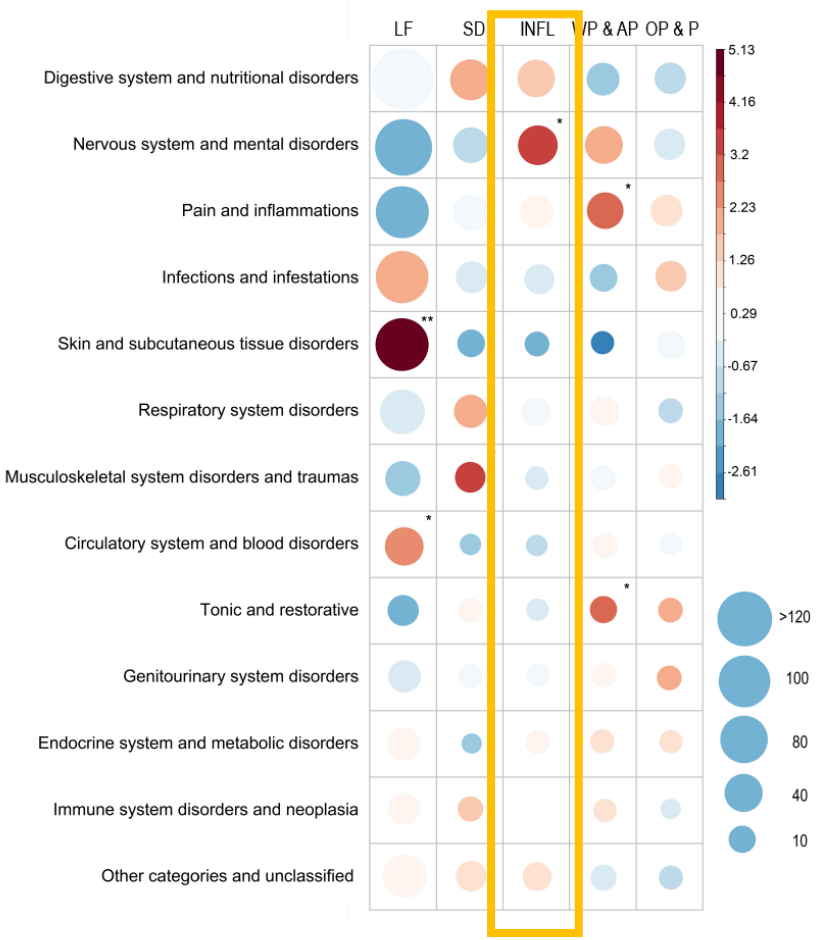
CANNUSE – A database of *Cannabis* traditional uses

- Digestive system and nutritional disorders
  - Laxative
  - Diarrheoa
  - Indigestion

- Musculoskeletal system disorders and traumas
  - Arthritis
  - Rheumatism

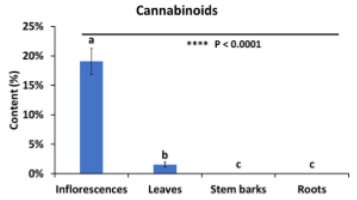
↪ antiarthritic, antirheumatic and analgesic properties ?

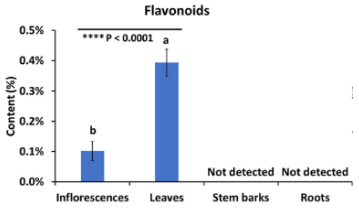
CANNUSE – A database of *Cannabis* traditional uses

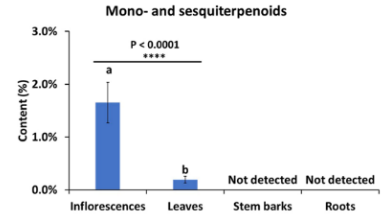


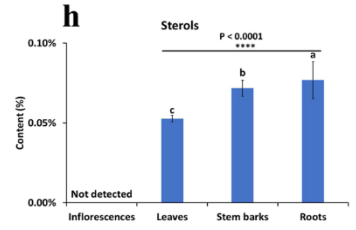
- Nervous system and mental disorders
  - Sedative ⟶ sedative properties

- Pain and inflammations





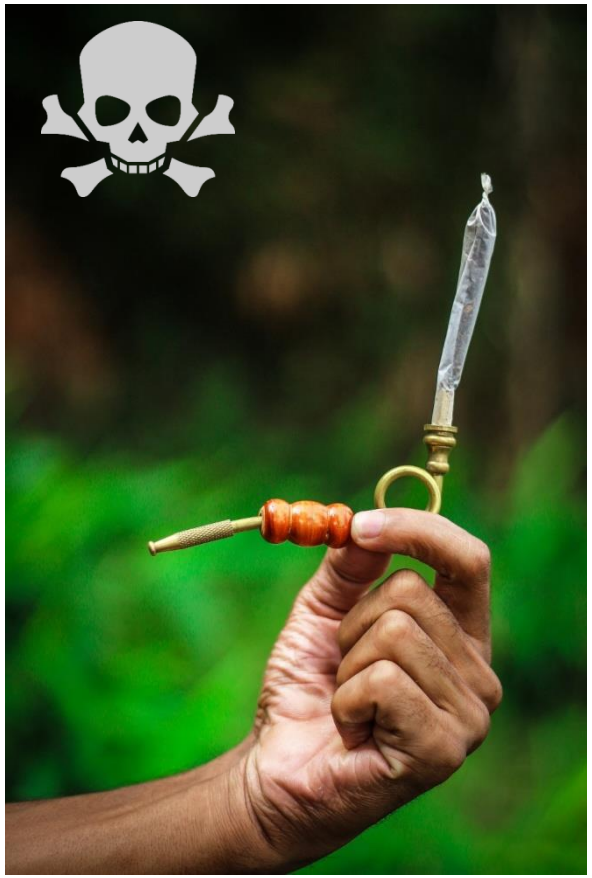




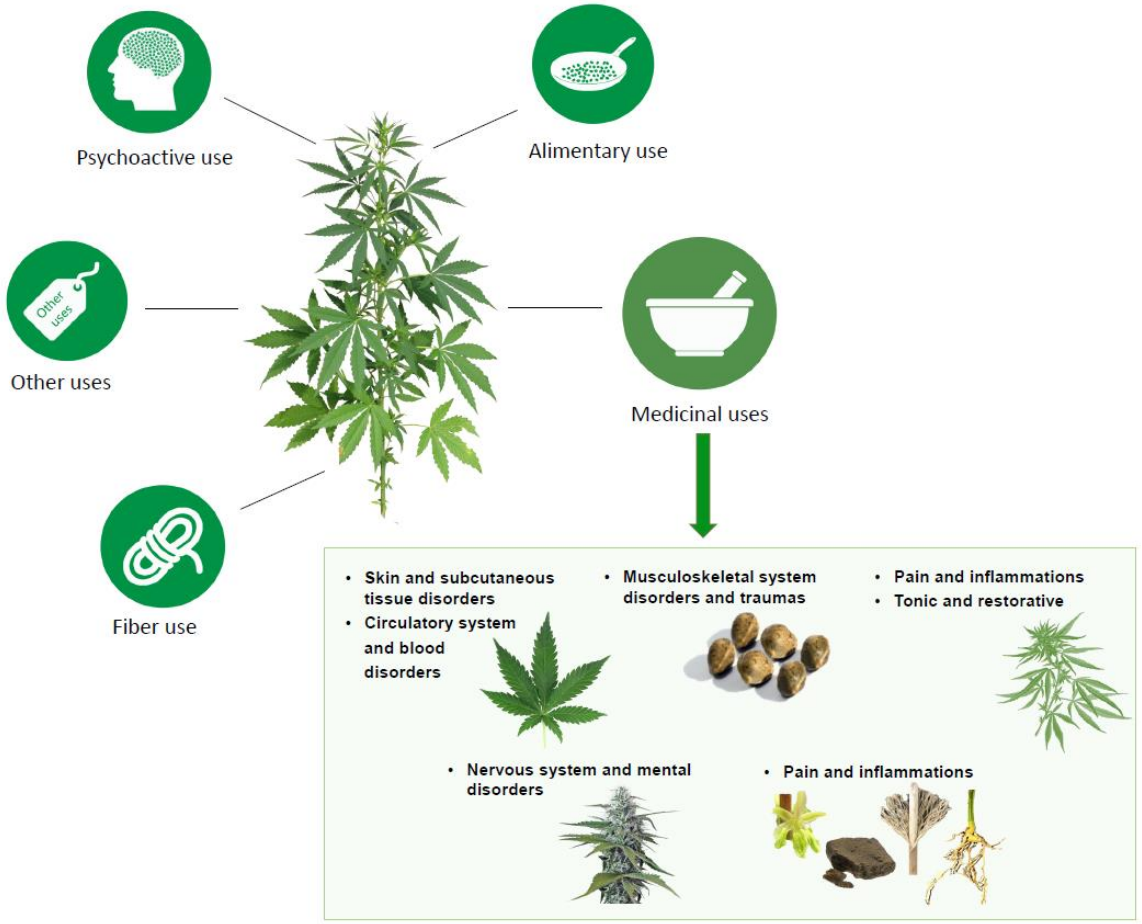
CANNUSE – A database of *Cannabis* traditional uses

Toxicity:

- Only 3.24% of references considered *Cannabis* toxic
  - Inflorescence (42.86%)
  - Leaf (40.82%)
- Only for prolonged and excessive use
- 45 unwanted effects
  - Hallucination
  - Poisoning
  - Drowsiness
  - Nausea
  - Vomiting
  - Death (in 1 reference)



CANNUSE – A database of *Cannabis* traditional uses



- In traditional medicine, all plant parts are used
- Clinical studies focus mostly on inflorescence use
- Future investigations should focus on **all parts of *Cannabis* plants!**

Balant et al. (2021) Traditional uses of *Cannabis*: An analysis of the CANNUSE database. Journal of Ethnopharmacology. https://doi.org/10.1016/j.jep.2021.114362

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Uriach

CIJA PRESERVATION

BFA BOTANICAL FARMING ALICANTE

- Herbarium Babes-Bolyai University, Cluj
- Herbarium Moscow State University, Moscow
- Herbarum Botanical Garden-Institute of FEB RAS, Vladivostok
- Herbarium Altai State University, Barnaul
- Herbarium Lund University, Lund
- Herbarium Institute of Botany, Chinese Academy of Sciences, Beijing
- Herbarium Institut Botànic de Barcelona, Barcelona

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