Development of the Industrial Chain for the Production of Cannabigerol (CBG): from Plant to Pure Compound

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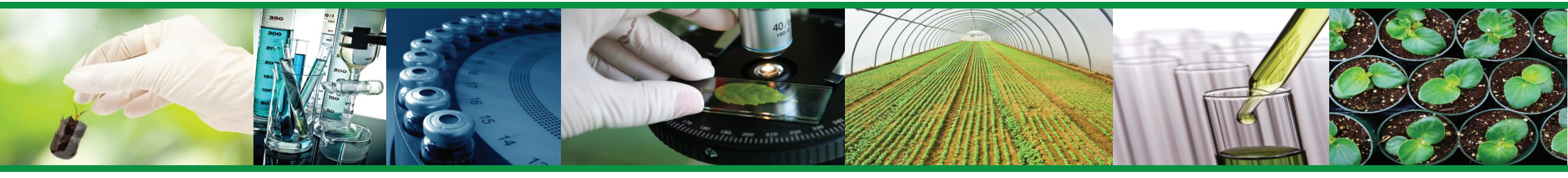

PHYTOPLANT RESEARCH
www.phytoplant.es

Cann 10
Tel Aviv, Israel
June 6 2017

COMPANY INTRODUCTION

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- Phytoplant Research S.L. is a privately-owned Spanish company founded in 2008 and active in the field of phytotherapy research.
- The company specializes in developing the industrial chain of medicinal plants, from selection and breeding to registration (for example, at Community Plant Variety Office (CPVO)), cultivation of registered varieties and obtaining derived products.
- Phytoplant Research S.L. focuses on research and development products that contain plant material and extracts, as well as essential and seed oils. These products represent significant economic potential for the pharmaceutical, nutraceutical and dermocosmetic industries.
- The company also wants to ensure the industrial supply of phyto-pharmaceutical quality raw materials through the cultivation of medicinal plants and extraction of plant material, with the objective of isolating, purifying and manufacturing naturally active ingredients (psychotropic and non psychotropic), semi-synthetic, biotransformed, pharmaceutically acceptable salts and derivatives.
- Phytoplant Research S.L. is committed to the quality, safety and innovation of the full range of its products and services:

SPECIALIZATION AREAS

- Selection and cultivation of medicinal plants
- Extraction of plant material
- Following best industry standards and certifications
- Consulting and technical advice on crop industrialization

Métodos de extracción y purificación

- **Proceso de producción – extracto / cannabinoides purificados**

CULTIVO → **SECADO** → **PROCESADO** → **EXTRACCION** → **PURIFICACION**

CBD

CBGA

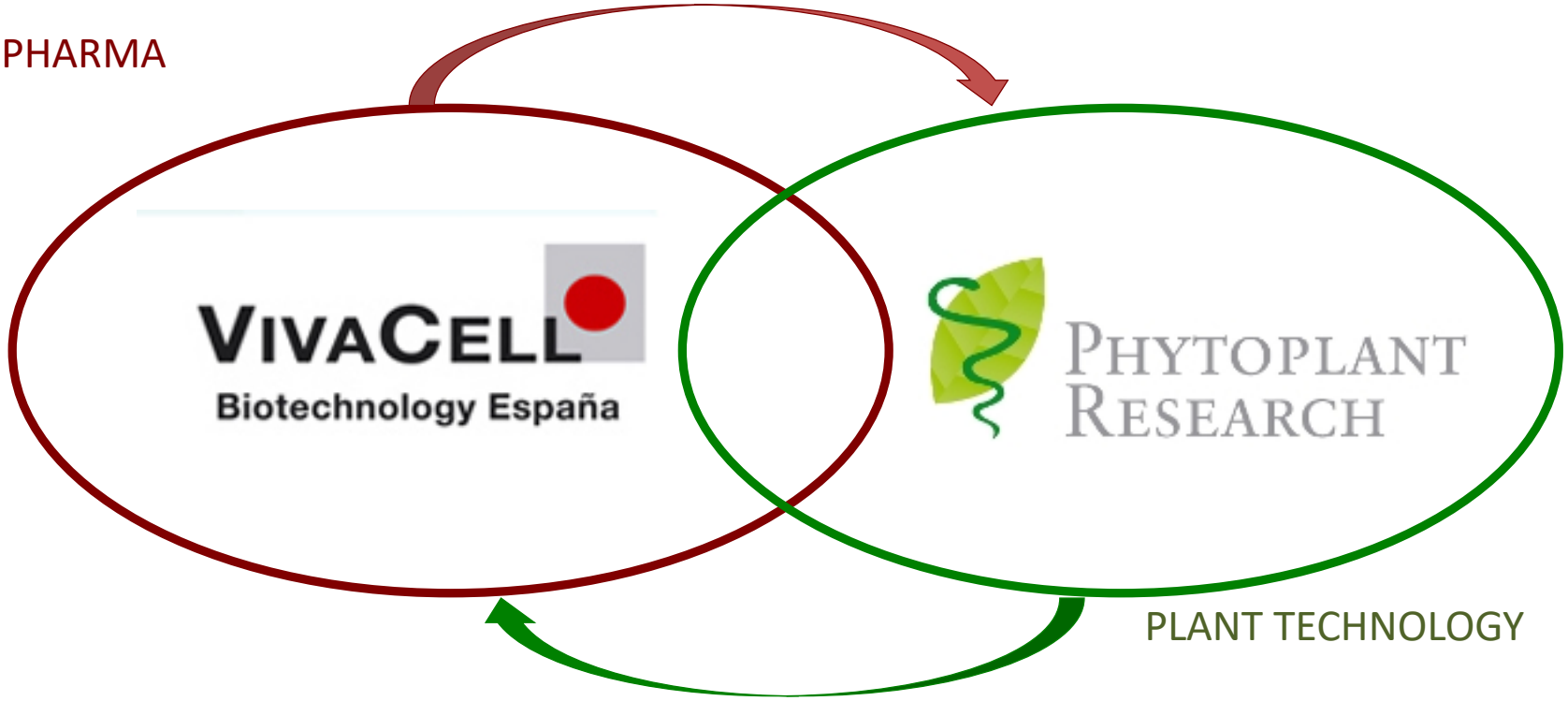
CBG

Good Agricultural Practices GAP

GOOD MANUFACTURING PRACTICE GMP

INTRODUCTION

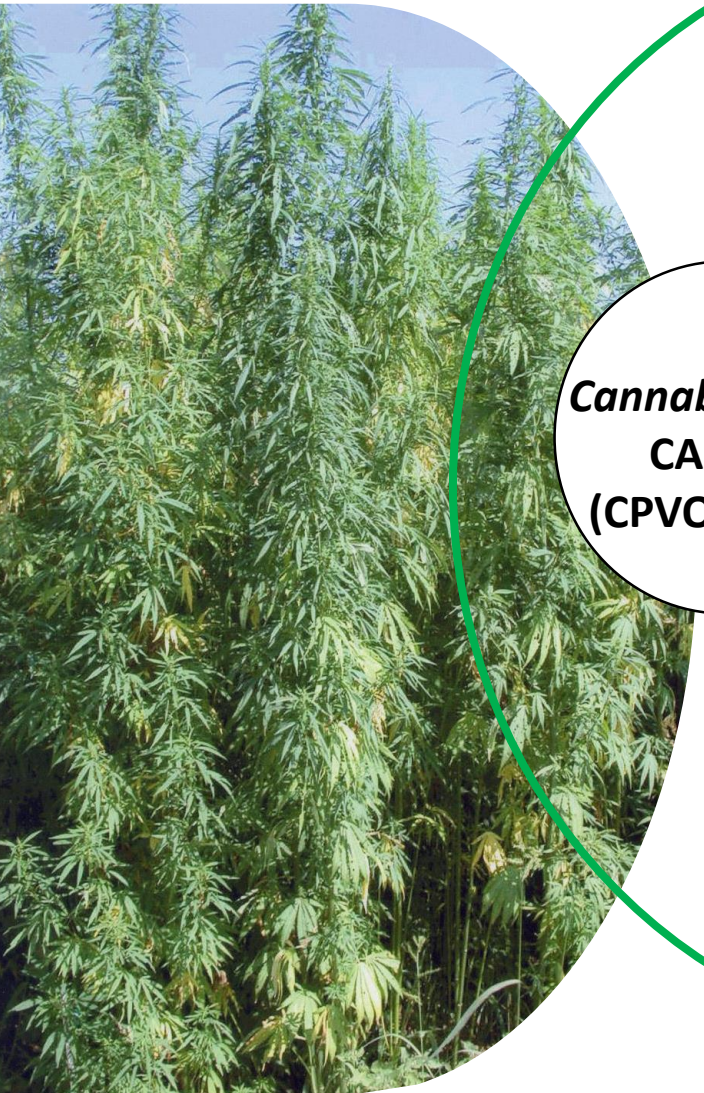
- **Joint Venture with Vivacell Biotechnology España since 2012:**



Vivacell, is the owner of the monoecious hemp variety registered at the CPVO (file nº 16305) identified with the denomination CARMA (chemotype CBG 3% (max) - THC<0,2%) and the rights of explotation have been cedited to Phytoplant.

INTRODUCTION

- **The strategy of Vivacell is to improve the biological activity of the phytocannabinoids and ensure the intellectual property of the NCE**



PHYTOPLANT RESEARCH S.L.

Cannabis sativa CARMA (CPVO 16305)

EXTRACTS

HERBAL DRUG

CDE-001

ATOPIC DERMATITIS
SKIN INFECTIONS

PHYTOCANNABINOIDS AS A CHEMICAL PRECURSORS

CBG

CBD

VCE-003

HUNTINGTON
PARKINSON
LATERAL AMIOTROPHIC SCLEROSIS

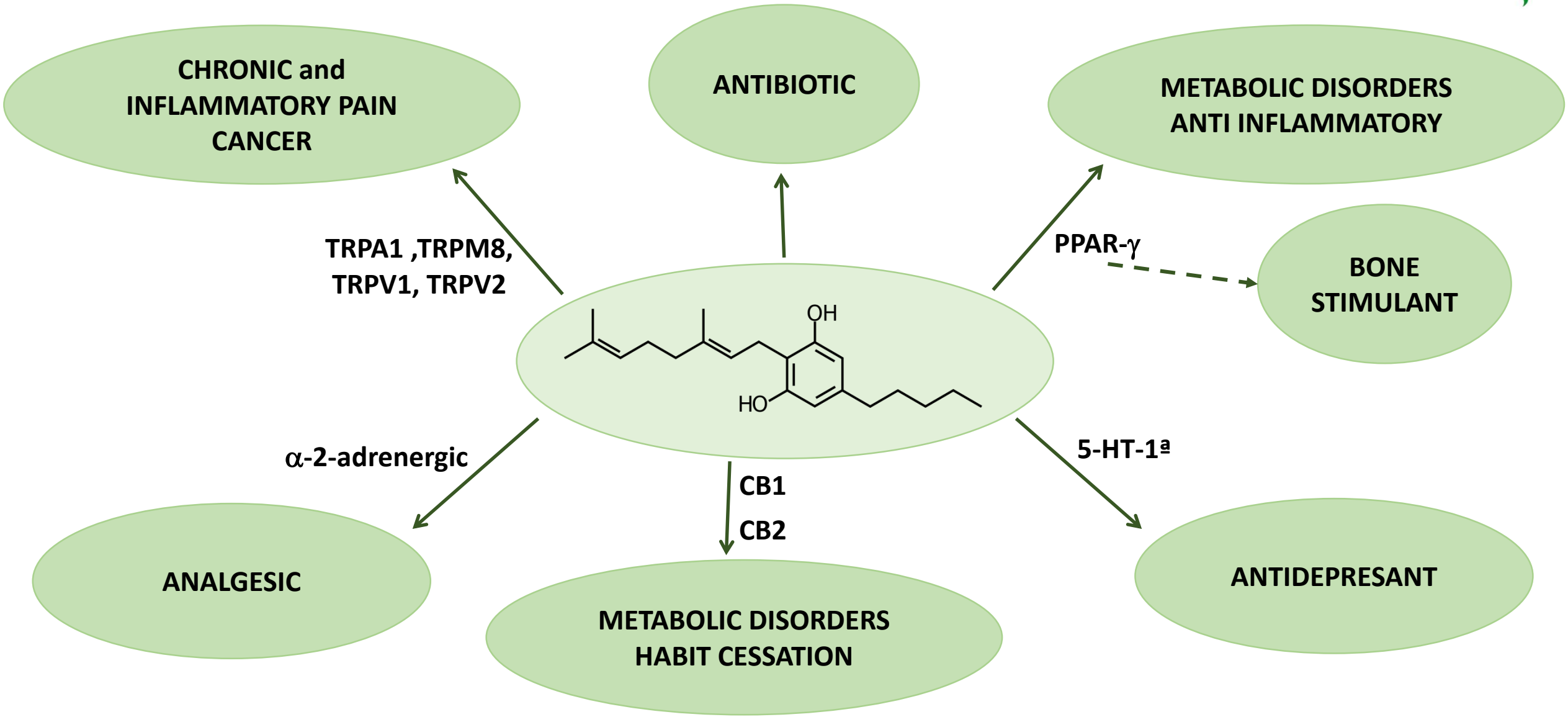
VCE-004

SCLEROSIS MULTIPLE
SCLERODERMIA

NEW CHEMICAL ENTITIES (NCE)

INTRODUCTION

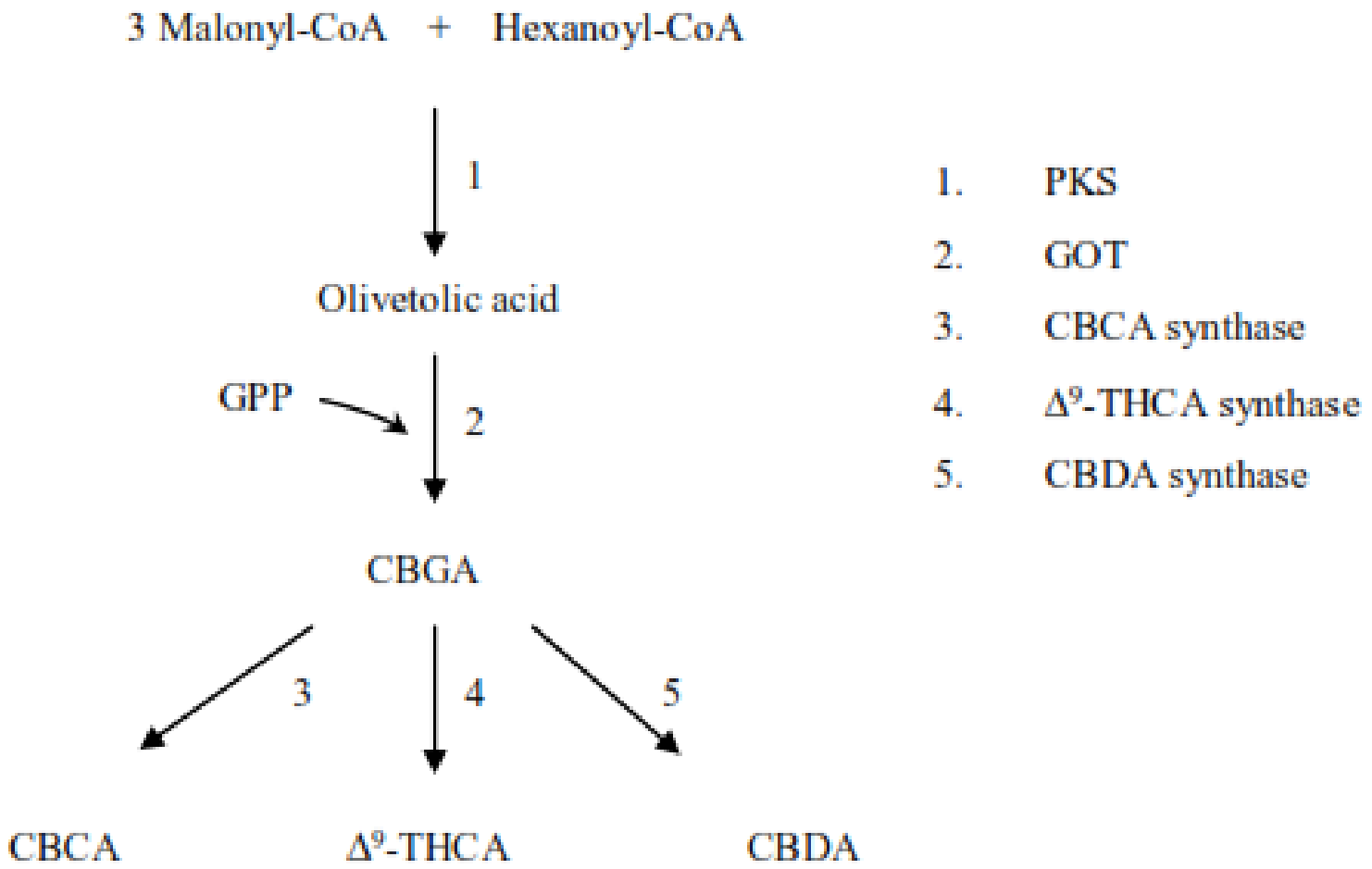
- **Mechanism of action and effects of CBG**



(Cascio et al., 2009) (De Petrocelis et al., 2010) (Granja et al., 2012)

INTRODUCTION

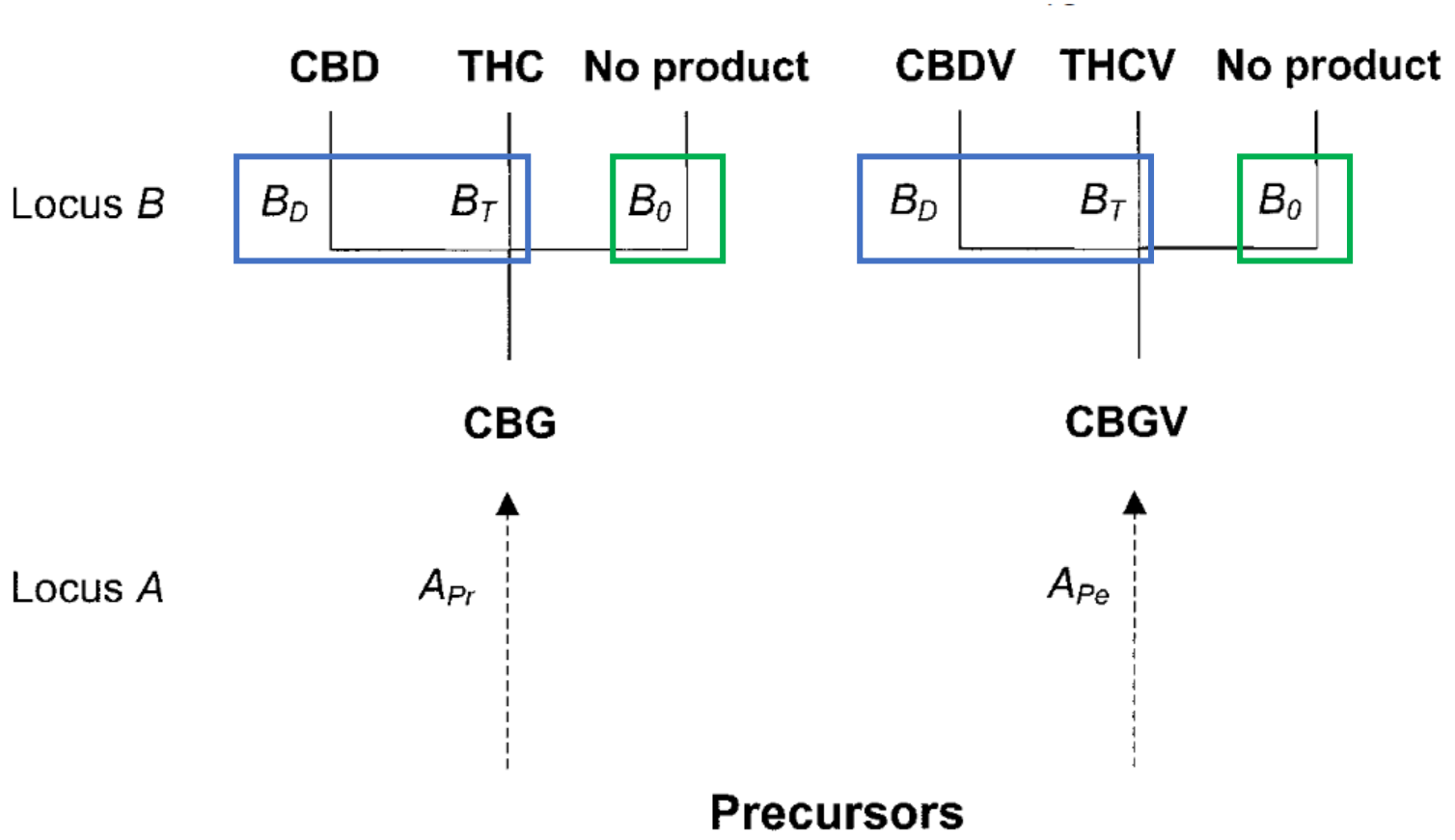
- **Biosynthesis of cannabinoids in *Cannabis sativa* L.**



(Flores Sanchez & Verporte, 2009)

INTRODUCTION

- **Genetic theory of cannabinoid biosynthesis:**



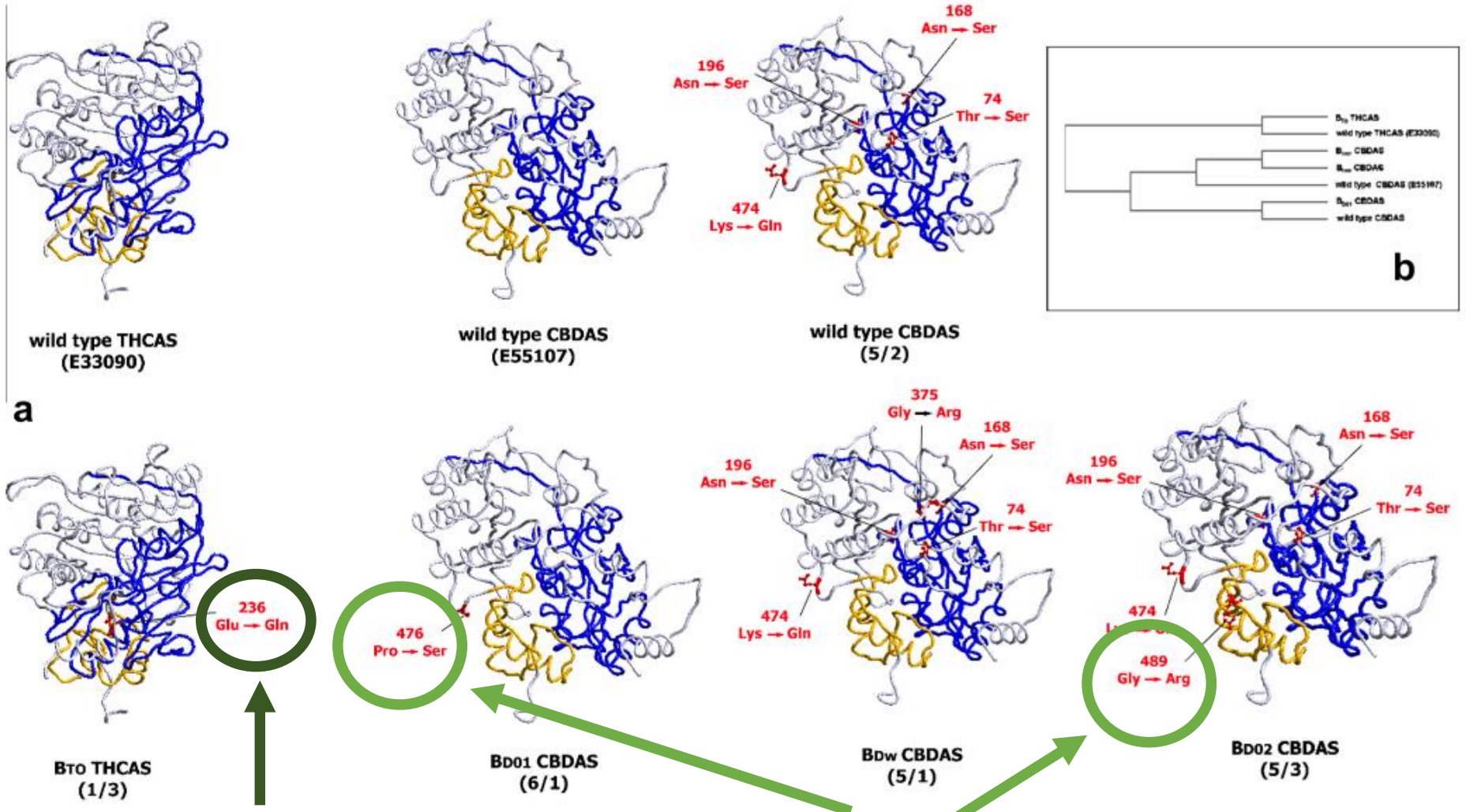
Codominant Alleles encoding for THCAS (B_T) or CBDAS (B_D)

Rececive Allele that encodes for a non functional CBDAS (B_0) that leads to CBG accumlation

(de Meijer, et al. 2003)

INTRODUCTION

- **Heterogeneity in the Cannabinoid synthases encoded in the Locus B:**



Mutation that produces a non functional THCA synthase

Mutations that produces a non functional CBDA synthase

Onofri et al. (2015)

BREEDING OF CANNABIS VARIETIES OF CHEMOTIPE IV (CBG)

Phytoplant CBG varieties with protection at CVPO

Denomination: **CARMA**
CPVO file N: **2003/0046**
CPVO grant N: **16305**
Sex expression: **mainly monoecious, but presence of dioecious male and female plants**
Propagation: **sexual (by seeds)**
Chemotype: **CBG (most of the plants)**
CBG: **1,5 – 3,0%**
Yield: **2 - 5 tons/Ha** (flowers and leaves)
Cultivation in open field

Denomination: **CARMA C80**
Sex expression: **dioecious female plant**
Propagation: **asexual (by cuttings)**
Chemotype: **CBG**
CBG: **1,5 – 3,0%**
Yield: **250 - 300 gr/planta** (flowers and leaves)
Cultivation in high tunnel

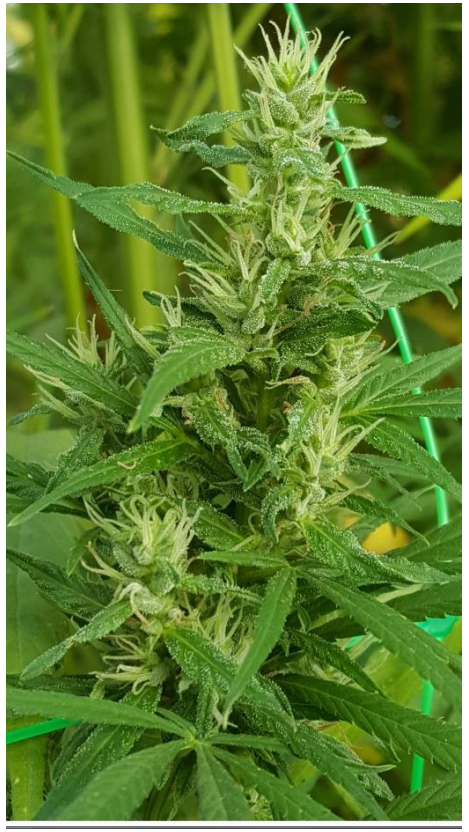
Denomination: **CARMA C54**
Sex expression: **dioecious female plant**
Propagation: **asexual (by cuttings)**
Chemotype: **CBG**
CBG: **1,5 – 3,0%**
Yield: **225 - 275 gr/planta** (flowers and leaves)
Cultivation in high tunnel

BREEDING OF CANNABIS VARIETIES OF CHEMOTIPE IV (CBG)

Phytoplant CBG varieties with provisional protection at CVPO

Denomination: **AIDA**
CPVO file N: 2016/0167
Chemotype: **CBG**
CBG: **3,5 - 8,5%**
Yield: **500 - 700 gr/plant**
(flowers and leaves)
Cultivation in high tunnel

Denomination: **JUANI**
CPVO file N: 2016/0117
Chemotype: **CBG+CBGV**
CBG: **2,0 - 4,5%,** CBGV: **0,3-0,6%**
Yield: **700 - 900 gr/plant**
(flowers and leaves)
Cultivation in high tunnel



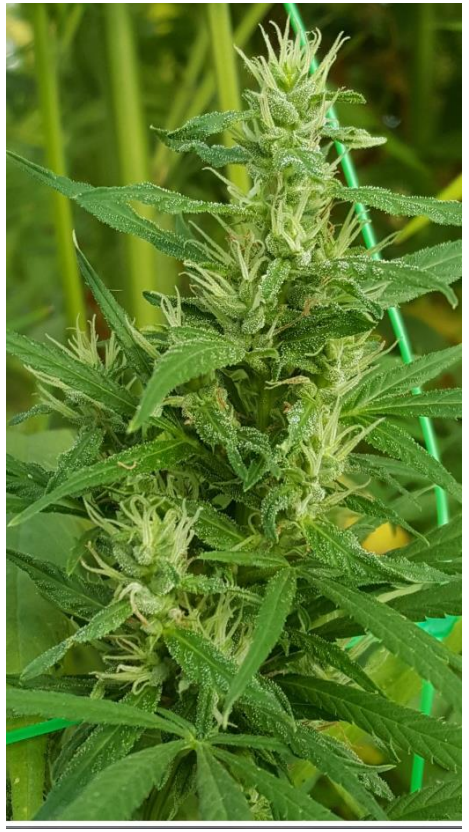
Denomination: **OCTAVIA**
CPVO file N: 2017/0148
Chemotype: **CBG**
CBG: **3,5 - 7,0%**
Yield: **700 - 900 gr/plant**
(flowers and leaves)
Cultivation in high tunnel

BREEDING OF CANNABIS VARIETIES OF CHEMOTIPE IV (CBG)

Phytoplant CBG varieties – other secondary metabolites

Denomination: **AIDA**
Major terpenoids: **Myrcene dominant**
Secondary terpenoids: **Guaiol : Phytol~ 1 > β Caryophyllene : α Pinene~ 1**
Cannflavin A: **470.3 µg/g**
Cannflavin B: **292.6 µg/g**
Canniprene: **5.5 µg/g**

Denomination: **JUANI**
Major terpenoids: **Phytol dominant**
Secondary terpenoids: **Myrcene > β caryophyllene : guaiol~ 1 > α Pinene : Ocimene~ 1**
Cannflavin A: **316.3 µg/g**
Cannflavin B: **99.8 µg/g**
Canniprene: **11.2 µg/g**



Denomination: **OCTAVIA**
Major terpenoids: **Phytol dominant**
Secondary terpenoids: **Myrcene > β caryophyllene : Ocimene~ 1**
Cannflavin A: **106.0 µg/g**
Cannflavin B: **55.0 µg/g**
Canniprene: **81.3 µg/g**

CULTIVATION

- **Different Agro-technics studied as a function of variety and growing conditions**

CARMA is a sexually propagated (by seeds) monoecious variety

Open Field
(1 cycle/year)

High technology greenhouse
(4-5 cycles/year)

High Tunnel
(1 cycle/year)

Indoor CEA
(4-5 cycles/year)

CULTIVATION

- **Different Agro-technics studied as a function of variety and growing conditions**

Open Field
(1 cycle/year)

High technology greenhouse
(4-5 cycles/year)

High Tunnel
(1 cycle/year)

Indoor CEA
(4-5 cycles/year)

C54, C80, AIDA, JUANI and OCTAVIA
are asexually propagated (by cuttings) dioecious varieties

CULTIVATION

- **Yields of CARMA cultivated in open field from seeds**

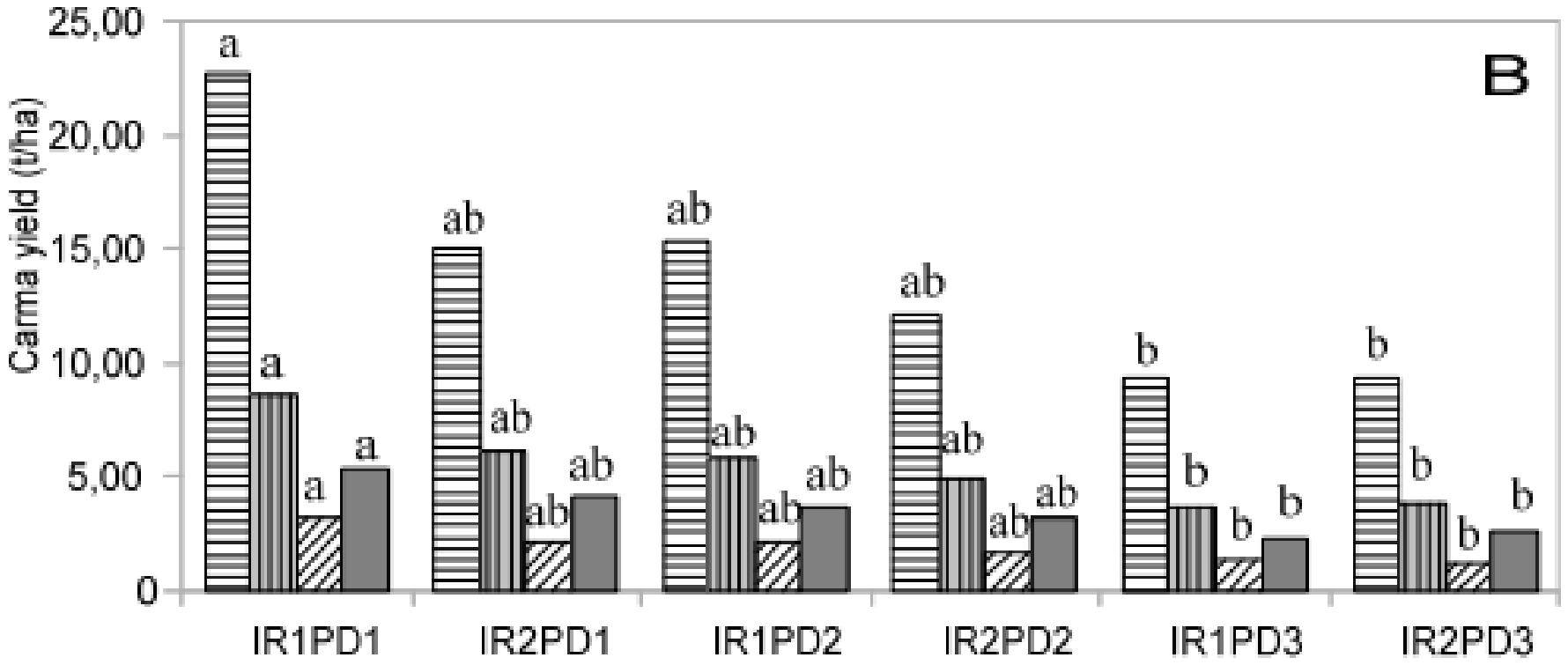
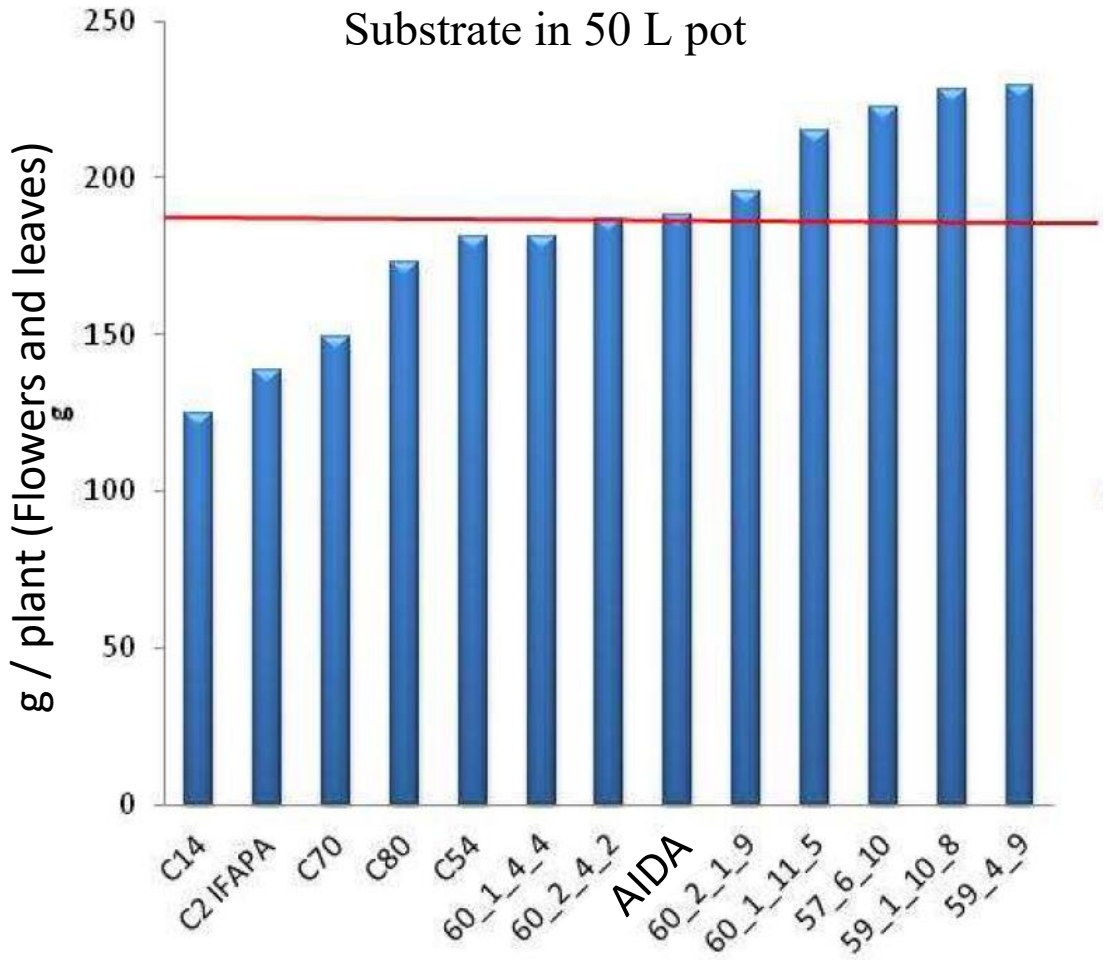


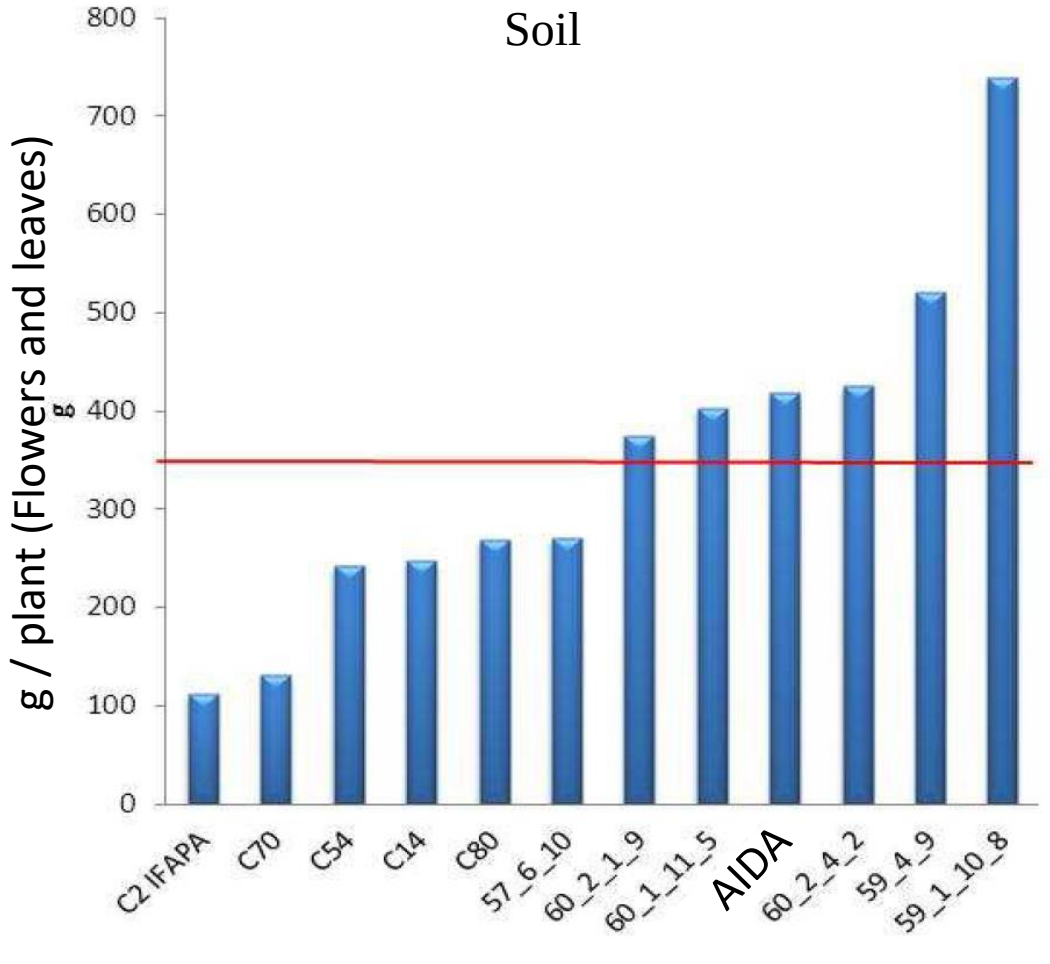
Figure 1. Yield values in Ermes (A) and Carma (B). Bars with different letters significantly differ ($p < 0.05$) by Tukey's test. IR_1, irrigated at 100% of crop evapotranspiration (ETc); IR_2, irrigated at 80% of ETc; PD, plant density; FW, fresh weight; DW, dry weight.

Garcia-Tejero et al. (2014)

CULTIVATION

- **Cultivation of experimental varieties in High tunnel. Comparison of yields in cultivation on pots or in the soil directly**





EXTRACTION & PURIFICATION OF CBG

Typical extraction/purification protocol of cannabinoids from *Cannabis sativa* L.

Step 1: Decarboxylation of PM (120 ° C for 1 hour).

Step 2: Extraction with supercritical fluid or solvent from PM

(WO2004026802A1, WO2002064109A2, WO2005000830A1 y WO2004016277A1)

Step 3: "Winterization" (Dissolve in EtOH and cooling to -20 ° C) of the extract

Step 4: Chromatography of the "winterized" extract to obtain the purified extract

Step 5: Dissolve the purified extract fractions in a first solvent (polar or non-polar), filter off any insoluble material, and remove the solvent from the filtrate

Step 6: Dissolve the filtrate in a second (non-polar or polar) solvent, filter off any insoluble material and remove the solvent from the filtrate to obtain a substantially pure cannabinoid

Step 7: Optional treatment with activated charcoal or Florisil

Step 8: Optional flash chromatography or recrystallization

Step 9: Optional Chemical Derivation and Crystallization

(Galal, et al. 2009)

EXTRACTION OF CBG

- **Basic methods of extraction of cannabinoids from *Cannabis sativa* L. plant:**

Extraction by infussion in water and milk or with solubilizers as cyclodextrins or lecitine.

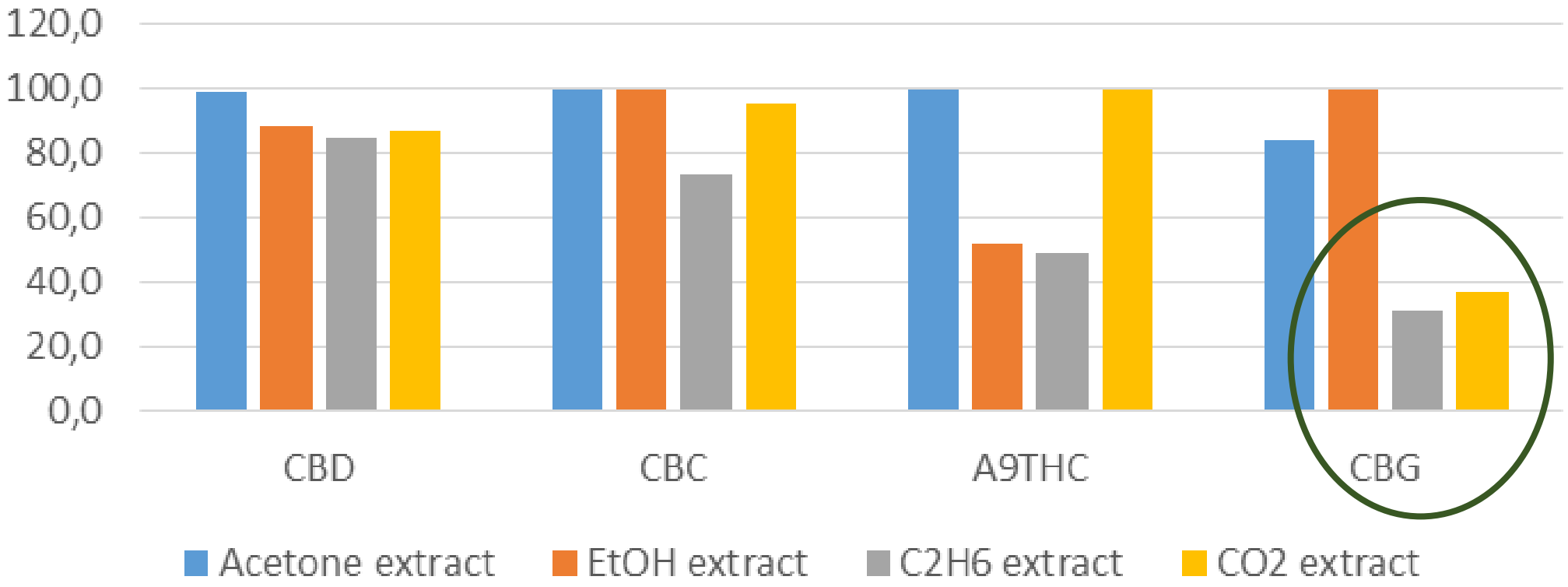
Extraction by direct maceration in food oil.

Extraction with liquid organic solvents:
- Extraction by percolation or maceration with organic solvents.
- Hot extraction with organic solvent, Soxhlet type.
- Auxiliar energy assisted extraction (Ultrasounds or Microwaves).

Extraction with pressurized gas:
- Extraction with CO2 in any state, liquid, subcritic or supercritical state.
- Extraction with butane or propane.
- Extraction with refrigerant gas.

EXTRACTION OF CBG

- **Solvent and extraction technique comparative in the yield in % of extraction of cannabinoids from *Cannabis sativa* L.**



PURIFICATION OF CBG

- **Basic methods of purification of cannabinoids from *Cannabis sativa* L. plant:**

Purification by molecular distillation.

Purification by derivates formation and recristallization.

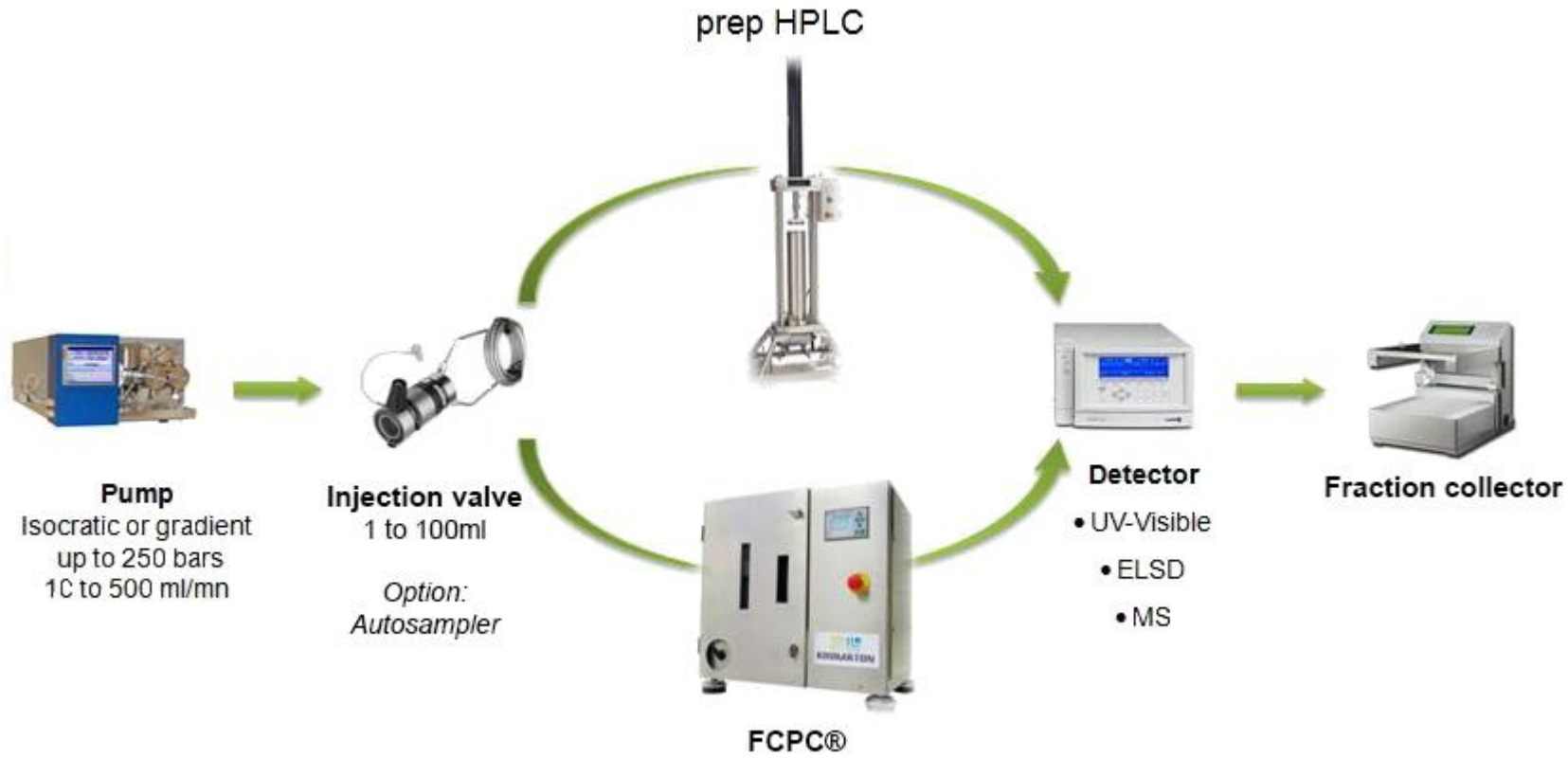
Purification by solid-liquid chromatography methods :
- Purification by column cromatography (Silica, C18 or other solid phases) by gravity or Flash.
- Purification by high pressure liquid chromatography (HPLC) in column of Silica, C18 or other solid phases.
- Purification by supercritical chromatography (SFC) using CO_2 as a solvent.

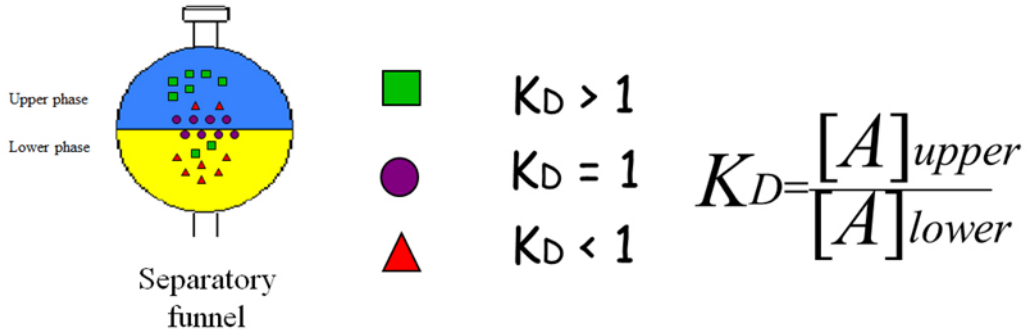
Purification by liquid-liquid chromatography methods:
- Purification by centifugal partition chromatography (CPC).
- Purification by counter courrent chromatography (CCC).

Purification by recristallization.

PURIFICATION OF CBG

- **Purification by liquid-liquid chromatographic methods (CPC & CCC):**

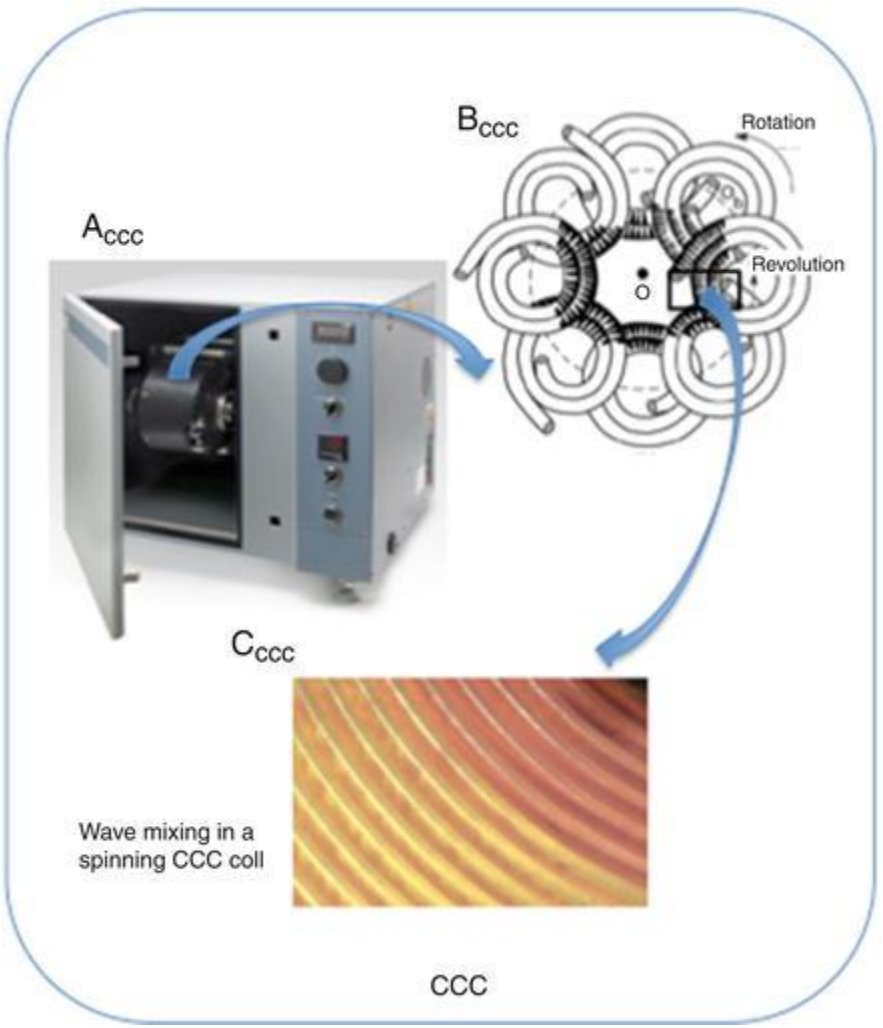


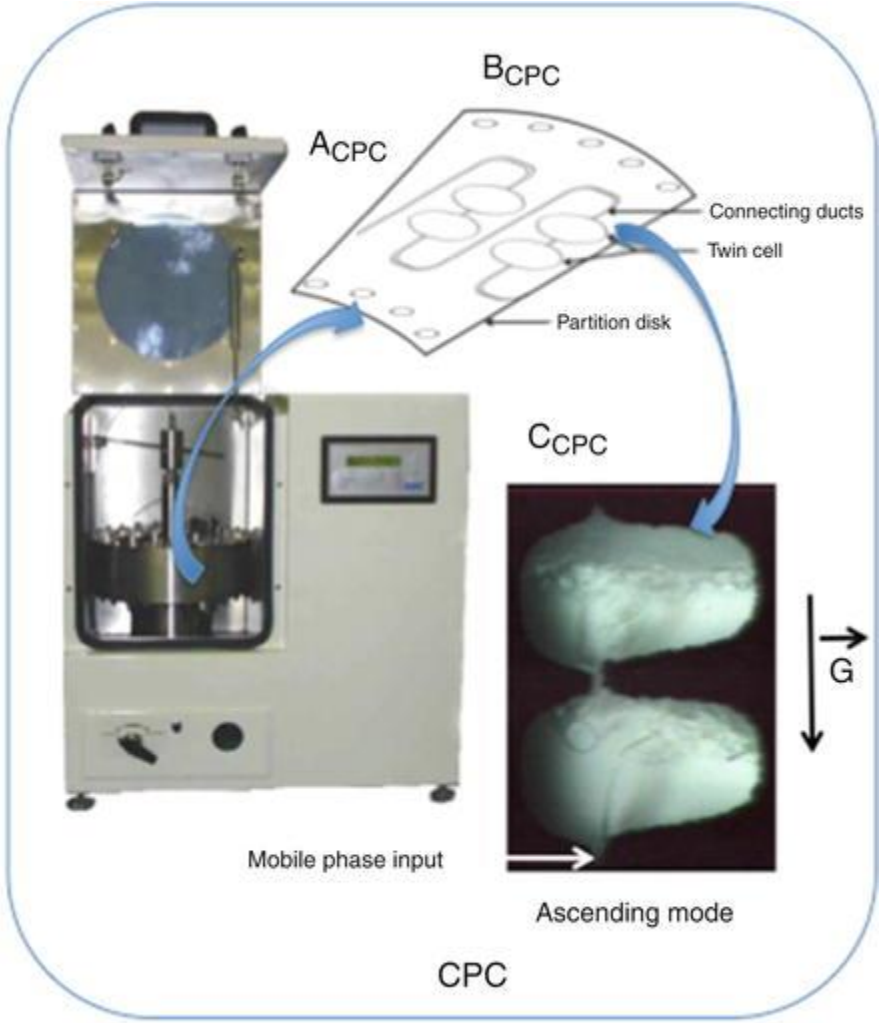


$$K_D = \frac{[A]_{upper}}{[A]_{lower}}$$

PURIFICATION OF CBG

- **Purification by liquid-liquid chromatographic methods (CPC & CCC):**





PURIFICATION OF CBG

- **Comparation of cannabinoid purity of isolated cannabinoids purifyied by liquid-liquid chromatographic methods (CPC Vs CCC):**

Isolated cannabinoid	Isolated in this study (mg)	Relative yield [a)]	purity GC [b)]
Δ^9-THC	90,0	0,83	93.1%
THCA	1590	8,34	94.0%
CBD	232	0,46	92,7%
CBDA	326	0.65	90,2%
CBG	40,3	0,54	92,2%
CBGA	37,9	0,46	92,9%
CBN	99,4	1,38	95,0%

(Hazekamp et al., 2004)

Compuesto	Concentración (% peso seco ± SD)
CBD	98,76 ± 0.45
CBDV	100.00 ± 206
CBDA	98.24 ± 2.81
THCA	98.14 ± 0.12
THC	95.04 ± 0.18
CBGA	95,88± 0.28
CBG	99.06± 0.08

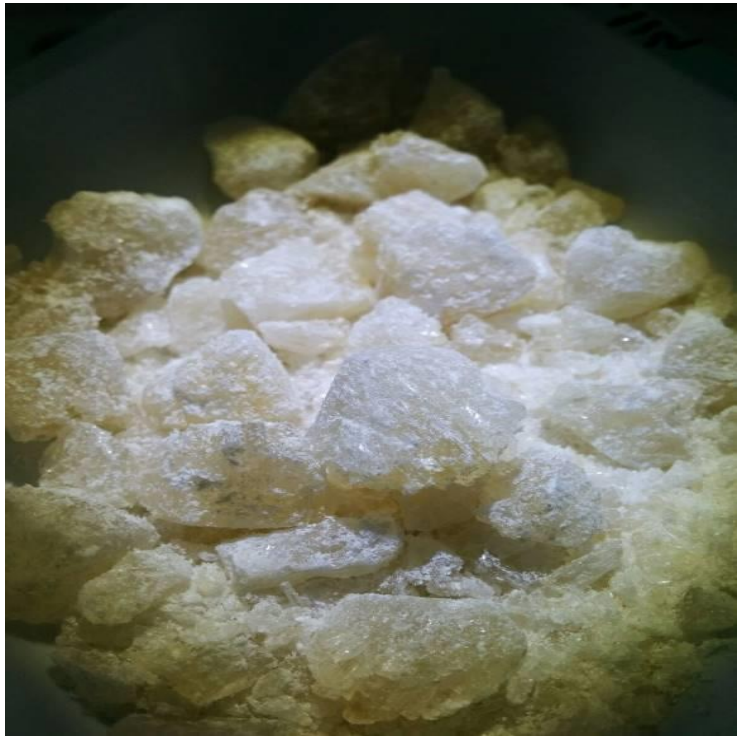
(Internal data of Phytoplant Research SL)

PURIFICATION OF CBG - Purification Patent USPO-PCT

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
15/004,848	01/22/2016	1629	730	3IPPP1.0001US	20	2



Purification patent "**Methods of Purifying Cannabinoids, Compositions and Kits Thereof**"
US 20160214920 A1

- **Easy to Scale Up method**
- **Non expensive equipment**
- **Quick and efficient method**
- **Environmentally friendly**

PURIFICATION OF CBG - Purification Patent USPO-PCT

- **Phytoplant patent PCT / EP2016 / 051388: METHODS OF PURIFYING CANNABINOIDS, COMPOSITIONS AND KITS THEREOF**

Step 1: Incubating the PM with non-polar solvent

Step 2: Reducing the volume of the 1st solvent mixture to about 50%

Step 3: Incubating the reduced 1st solvent mixture (-70-40ºC) to crystalize the cannabinoids

Step 4: Dissolving the crystalized cannabinoids with non-polar solvent (being the 2nd solvent mixture)

Step 5: Incubating the 2nd solvent mixture (-70-40) to crystalize the cannabinoids. Getting a purification of cannabinoids > 95%.

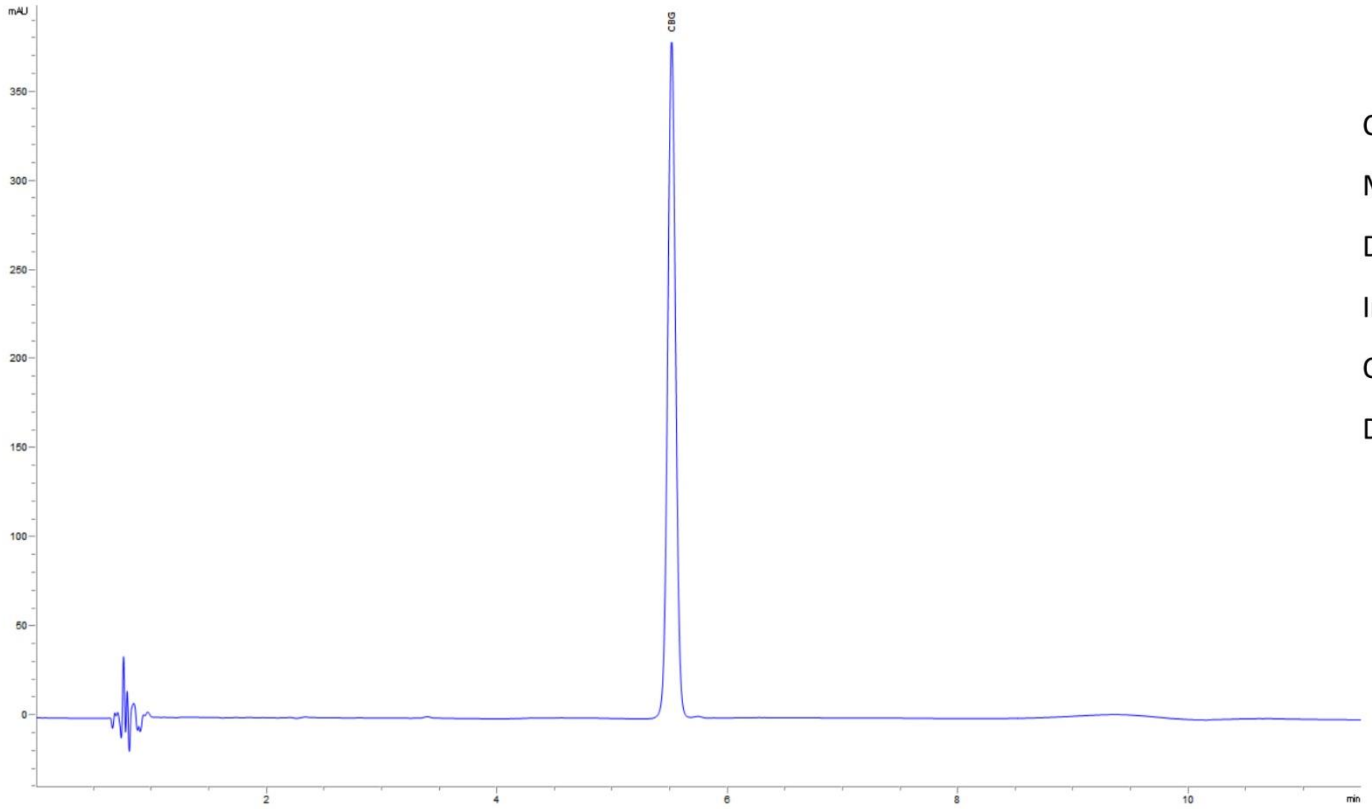
TESTING AND QUALITY CONTROL

- Sampling
- Identification
- Chemical determinations
  - Foreign matter
  - Water quantification
  - Total ash quantification
  - Acid insoluble ash quantification
  - Determination of the purity of the APIs (cannabinoids)
  - Quantification of mycotoxins (aflatoxins and ochratoxins)
  - Determination of heavy metals
  - Determination of residual solvents
  - Determination of pesticides
- Microbiological determination

TESTING AND QUALITY CONTROL

Determination of the purity of the APIs (cannabinoids)

Name	Component	Purity (%)
CBG > 98 %	Cannabigerol (CBG)	99.67 ± 0.46



Column: InfinityLab Poroshell, Ec-C18, 2.7µm size particule, 150 x 2.1mm

Movil phase: Water and Acetonitrile with formiate ammonium

Det.: DAD, 210nm

Inj.: 3 µL

Oven: 30 ºC

Date: 25/08/2016

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Thank you for your attention