BREEDING AND AGRONOMICAL TECHNIQUES TO PRODUCE MEDICAL CANNABIS

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Italian pioneers of *Cannabis sativa* L. breeding

**Dr. Domenico Allavena**  
(Bologna 1910-1985)

Obtained the varieties:  
- Fibranova and  
- Carmagnola Selezionata  
  (C.S.)

On the left an original picture made by Allavena of the *Pinnatofidofilla*
Hemp Cultivation Area in the EU (ha) 2016: ca. 33,300 ha
Italian Regions (12/20) that have approved the law that support the cost of the drugs with natural or synthetic cannabinoids.

- Puglia,
- Tuscany
- Liguria,
- Veneto,
- Friuli Venezia Giulia
- Abruzzo
- Umbria
- Marche
- Sicily
- Emilia Romagna
- Trento and Bozen Aut. Reg.
- Piedmont
Medicinal Cannabis origin

*Cannabis sativa* L.: Dioecious plant

Female flower
Cannabis accessions collected in Italy  
(more than 300 strains)
Cannabis germplasm collection in Italy

Ind. Hemp: 153
Landraces: 68
Drug type: 163
Some example of genotypes with interesting characters
The only one area in open field admitted in Italy to grow Cannabis with high THC concentration (2013-2018) about 2,000 sqm
Cannabis genotypes with high THC grown in 2016
Breeding method is based on reversion of sex with Ag salt.
Natural photoperiod  Controlled photoperiod
Results achieved by the most advanced breeding by the leading pharmaceutical company in the Cannabis sector

<table>
<thead>
<tr>
<th>Chemotype (main cannabinoid)</th>
<th>Clone (code)</th>
<th>BRM (g/m²)</th>
<th>$C_{tot}$ (%w/w)</th>
<th>Target cannabinoid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Purity (%w/w)</td>
</tr>
<tr>
<td>CBG</td>
<td>M378</td>
<td>792</td>
<td>11.2</td>
<td>99.9</td>
</tr>
<tr>
<td>CBGV</td>
<td>M350</td>
<td>507</td>
<td>10.4</td>
<td>87.4</td>
</tr>
<tr>
<td>THC</td>
<td>M87</td>
<td>650</td>
<td>15.3</td>
<td>96.8</td>
</tr>
<tr>
<td>THCV</td>
<td>M264</td>
<td>609</td>
<td>14.5</td>
<td>81.7</td>
</tr>
<tr>
<td>CBD</td>
<td>M255</td>
<td>810</td>
<td>14.5</td>
<td>88.7</td>
</tr>
<tr>
<td>CBDV</td>
<td>M276</td>
<td>475</td>
<td>9.5</td>
<td>71.0</td>
</tr>
<tr>
<td>CBC</td>
<td>M394</td>
<td>731</td>
<td>2.9</td>
<td>93.4</td>
</tr>
<tr>
<td>CBCV</td>
<td>M206</td>
<td>283</td>
<td>1.8</td>
<td>52.6</td>
</tr>
<tr>
<td>Cannabinoid-free</td>
<td>M299</td>
<td>620</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

Chapter 5
The Chemical Phenotypes (Chemotypes) of Cannabis
Etienne de Meijer

Research programs running at the CREA- CI

1. Cannabis without cannabinoids
2. Cannabis with chemotype CBG prevalent
3. Cannabis with chemotype CBD prevalent
4. Cannabis with chemotype THC prevalent
5. Cannabis with chemotype THCV prevalent
6. Cannabis with chemotype CBDV prevalent
7. Cannabis with high content of GLA in seed
8. Cannabis with selected terpenes
9. Cannabis with high yield for indoor and outdoor condition.
Gas-chromatogram of Ermo’s extract
The analysis of Ermo variety could be done using simple test

A colorimetric test allows to identify contamination
Chemotype of Cannabis strain with THC prevalent

* = Internal Standard 0.01% conc.
Variability of THC content we have available in our accessions

Apical flowers, 2 samples per plant.
Mother plants of CINBOL variety registered in the CPVO and used as medicinal cannabis by the Italian state

Cuttings in rooting phase
Chemotype of Cannabis strain with CBD prevalent
Production of CBD in varieties of Italian cannabis grown in pots

*** Uso31 and Santhica, THC = 0
Chemotype of Cannabis strain with CBG prevalent
Chemiotipo di Cannabis con CBDV/CBD prevalente
Chemotype of Cannabis strain with THC/THCV prevalent
How to grow Cannabis?

Indoor, hydroponic

Vertical farming

SCROG
Light Treatments

- Valoya NS1 had the highest portion of blue light, including some UVA light.
- Valoya AP673L spectrum had the most red light, spectrum designed for high vegetative growth.
- HPS spectrum concentrated in the yellow-orange area of the visible spectrum. Very low blue light content.

<table>
<thead>
<tr>
<th>Range</th>
<th>HPS</th>
<th>AP673L</th>
<th>NS1</th>
</tr>
</thead>
<tbody>
<tr>
<td>300-400</td>
<td>1%</td>
<td>0%</td>
<td>2%</td>
</tr>
<tr>
<td>400-500</td>
<td>8%</td>
<td>14%</td>
<td>24%</td>
</tr>
<tr>
<td>500-600</td>
<td>68%</td>
<td>20%</td>
<td>37%</td>
</tr>
<tr>
<td>600-700</td>
<td>21%</td>
<td>59%</td>
<td>33%</td>
</tr>
<tr>
<td>700-800</td>
<td>3%</td>
<td>7%</td>
<td>4%</td>
</tr>
<tr>
<td>PAR 400-700</td>
<td>96%</td>
<td>93%</td>
<td>94%</td>
</tr>
<tr>
<td>R:FR</td>
<td>2.80</td>
<td>6.07</td>
<td>10.05</td>
</tr>
<tr>
<td>B:G</td>
<td>0.29</td>
<td>1.76</td>
<td>0.74</td>
</tr>
<tr>
<td>B:R</td>
<td>0.10</td>
<td>0.26</td>
<td>0.80</td>
</tr>
</tbody>
</table>
No significant differences found in the total yield of cannabinoids between the light treatments or between the two experiments.

When comparing the yield of cannabinoids per Watts of electricity used, LED treatments had the best results in both trial rounds and HPS the lowest values.
OPERATIVE PHASE

Photoperiod
Vegetative 18/6
Generative 12/12

Day -15 0 18 42d 76 83 90
Cutting Transplant Change Photop. Flowering H2O Dry Harvesting
<table>
<thead>
<tr>
<th></th>
<th>33 Days</th>
<th>40 Days</th>
<th>46 Days</th>
<th>52 Days</th>
<th>56 Days</th>
<th>62 Days</th>
<th>69 Days</th>
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</thead>
<tbody>
<tr>
<td><strong>G170</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
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<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
</tr>
<tr>
<td><strong>G301</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
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<td><img src="image10.png" alt="Image" /></td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
<td><img src="image13.png" alt="Image" /></td>
<td><img src="image14.png" alt="Image" /></td>
</tr>
</tbody>
</table>
Cannabinoid concentration in the flowers during the plant cycle

Effect of position on total cannabinoids [%] content

Total Cannabinoids.

Flowering phase [days]

G170

G301

Top

Middle

Basal
Flower yield (dry weight)

Variety

<table>
<thead>
<tr>
<th>Variety</th>
<th>G-170</th>
<th>G-301</th>
</tr>
</thead>
</table>

Dry weight flowers [g/plant] vs. Flowering phase [days]

- Variety G-170
- Variety G-301
Accumulation of cannabinoids in Cannabis flowers
Mature plant

Principal fraction obtained

Leaves could be extracted with CO$_2$ SCF
Localization of the three fields where hemp with high CBD content has been grown.
Environment condition

Mean temperatures

<table>
<thead>
<tr>
<th>Month</th>
<th>Temp. °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feb</td>
<td></td>
</tr>
<tr>
<td>Mar</td>
<td></td>
</tr>
<tr>
<td>Apr</td>
<td></td>
</tr>
<tr>
<td>May</td>
<td></td>
</tr>
<tr>
<td>Jun</td>
<td></td>
</tr>
<tr>
<td>Jul</td>
<td></td>
</tr>
<tr>
<td>Aug</td>
<td></td>
</tr>
<tr>
<td>Sep</td>
<td></td>
</tr>
</tbody>
</table>

Legend:
- Blue: Lecce
- Red: Bari
- Green: Foggia
CBD concentration in the 3 fields

CBD (% dw)

Plant n°

Lecce
Foggia
Bari
Comparison between CBD and THC content

Cannabinoid variation

Concen. (% dw)

Lecce
Foggia
Bari

THC

CBD

5,5% CBD

0,20 % Limit

0,30

0,10

0,0

0,0
in vitro culture to improve breeding of Cannabis

Main tasks

1. Fast multiplication
2. Virus eradication
3. Long term storage

Micro-cuttings

Root phase

Pots with cannabis plantlets

Adaptation to normal condition
# Polyploidy strategy

<table>
<thead>
<tr>
<th></th>
<th>Diploid</th>
<th>Three-ploid</th>
<th>Tetra-ploid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell with</td>
<td>2n=2X=20</td>
<td>2n=3X=30</td>
<td>2n=4X=40</td>
</tr>
</tbody>
</table>

![Images of cells with different ploidy levels](images)
Polyploid lines of Cannabis are available

Three chemotypes have been used
- CBD
- CBG
- THC
First crop of medicinal Cannabis produced in Italy in the April 2015

Thank you for your attention!