

PhD School on Agriculture, Environment and Bioenergy

(http://sites.unimi.it/dottorato_aab/)

(XXXVI cycle, 2020-23)

Project draft

1. Field of interest

AGRI2

2. Project title

Cannabis cryptic virus new tool for the study of *Cannabis sativa* genome

3. Tutor (membro del Collegio dei Docenti)

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4. Relevance of the topic and state of the art:

Hemp (*Cannabis sativa*), represents a multi-purpose crop and is cultivated for fiber, seed, and oil and is characterized by a low THC content (Weiblen *et al.* 2015).

Seeds are particularly important because of their richness in essential and polyunsaturated fatty acids, amino acids, vitamins and minerals (Galasso *et al.* 2016). Hemp fiber cultivars are employed in order to obtain cellulosic and woody fibers for different productions, such as tissues and paper (Carus *et al.* 2013).

Lastly, hemp is a promising candidate for phytoremediation of contaminated soils (Campbell *et al.* 2002) owing to its fast-growth, high biomass, long tap root and metal tolerance.

The identification of the metabolic pathways involved in the synthesis of fatty acids, cannabinoids, and fiber production could be a substantial benefit in the genetic improvement of this crop.

The use of some biotechnological techniques, such as the use of viral vectors to induce silencing of genes in important metabolic pathways or cause the production of exogenous proteins, could lead to the selection of important genetic markers useful in the genetic improvement of *Cannabis* spp.

Cannabis sativa is naturally infected by many viruses; some of them are widespread and polyphagous such as *Cucumber mosaic virus* (CMV) or *Alfalfa mosaic virus* (AMV), while others are specific to this crop, such as *Sunn hemp mosaic virus* and *Sunn hemp leaf distortion virus*. A specific virus of particular interest is the *Cannabis cryptic virus*, a dsRNA virus that does not possess movement proteins and is not transmitted by any vector, making it potentially ideal for the construction of a viral vector.

5. Layout of the project (draft)

5.1. Materials & Methods:

The project will focus on the production of viral vectors starting from the *Cannabis cryptic virus* (CanCV), a dsRNA plant virus, to achieve two main goals: (i) verifying the etiology of the hemp streak disease, in which the involvement of this cryptic virus is suspected but not proved and (ii) induce virus-induced gene silencing (VIGS) to knock-out genes of interest in the genome of *Cannabis sativa* to understand the function of these genes.

Both goals will have an initial part in common in which the base of the viral vector, obtained in collaboration with the University of Bologna, will be tested for its ability to replicate in both a model host plant (*Nicotiana benthamiana*) and in the native host (*Cannabis sativa*), both in whole plants and in protoplasts.

The production of the viral vector modified for protein expression can follow different strategies: (i) the insertion of a ribosome-recognition sequence (Internal Ribosome Entry Sites IRES) to allow the translation of an additional coding sequence on the viral genome, or (ii) modifying one of the two proteins (polymerase or coat protein) to produce a polyprotein, fused with the protein of interest. This second approach will require the addition of an auto-catalytic site to obtain the separation of the two proteins after the translation process. It will be possible use viral vector for epitope expression of human virus in order to obtain a vaccine.

The production of the viral vectors modified to silence genes will be carried out by inserting in non-coding regions of the CanCV genome both sense and anti-sense sequences matching with *Cannabis* genes of interest, leading to dsRNA-mediated silencing of those genes and allowing to study the function of those genes. The effect of these silencing

constructs will be carried out on a collection of hemp made available by a collaboration with University of Bologna and CREA.

This analysis in functional genomics will attempt to identify key genes related to two different processes: the first is related to the production of Cannabis-derived drugs for the treatment of important diseases, such as epilepsy (Devinsky et al., 2014), by identifying the genes that can stimulate the production of cannabinoids; the second is to identify the genes that can lead to an improved nutritional value of hemp seeds, by reducing the phytic acid level in the seeds without reducing the overall quality and quantity of proteins and fatty acids contained in the seeds.

5.2. Schedule and major steps (3 years): **mezza pagina max**

First year

Inoculation of the viral vector in *N. benthamiana* and *C. sativa* plants, and verification of colonization

Study of relationship between CanCV and hemp streak disease

Production of protoplasts of *N. benthamiana* and *C. sativa*, and inoculation with the viral vector

Identification of *C. sativa* genes involved in pathways of interest

Second year

Modification of the viral vector for the expression of exogenous proteins

Modification of the viral vector for the silencing of genes of interest

Setup of a protocol for protoplast regeneration for *C. sativa*

Third year

Inoculation of the protoplasts with the modified viral vectors

Evaluation of the silencing efficacy through real-time or digital PCR

Protoplast regeneration

6. Available funds

GEMMA

6. Literature: **max 10 citazioni**

- Campbell S, Paquin D, Awaya JD, Li QX (2002) Remediation of benzo[a]pyrene and chrysene-contaminated soil with industrial hemp (*Cannabis sativa*). *Int J Phytoremediat* 4:157–168
- Carus M, Karst S, Kauffmann A, Hobson J, Bertucelli S (2013) The European hemp industry: cultivation, processing and applications for fibres, shivs and seeds. European Industrial Hemp Association, Hürth, Germany, pp 1–9.
- Devinsky O, Cilio MR, Cross H, Fernandez-Ruiz J, French J, Hill C, Katz R, Di Marzo V, Jutras-Aswad D, Notcutt WG, Martinez-Orgado J, Robson PJ, Rohrback BG, Thiele E, Whalley B, Friedman D (2014) Cannabidiol: pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. *Epilepsia* 55:791–802
- Galasso I, Russo R, Mapelli S, Ponzoni E, Brambilla IM, Battelli G, Reggiani R (2016) Variability in seed traits in a collection of *Cannabis sativa* L. genotypes. *Frontiers Plant Sci* 7:688
- Weiblen GD, Wenger JP, Craft KJ, ElSohly MA, Mehmedic Z, Treiber EL, Marks MD (2015) Gene duplication and divergence affecting drug content in *Cannabis sativa*. *New Phytol* 208:1241–1250