

Breeding, genetics and genomics of industrial hemp: the CaRiFIT2022 project

6.26

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Background

The project **CaRiFIT2022 (Canapa e Ricerca Filiera Italiana 2022)**, <https://carifit.crea.gov.it/> is a 5-year national research and development project (2023-2028), sponsored by the Italian Ministry of Agriculture, Food Sovereignty and Forests (MASAF). It tackles some of the main problems of the national hemp seed production chain still preventing it to be fully competitive and rewarding for the Italian hemp growers.

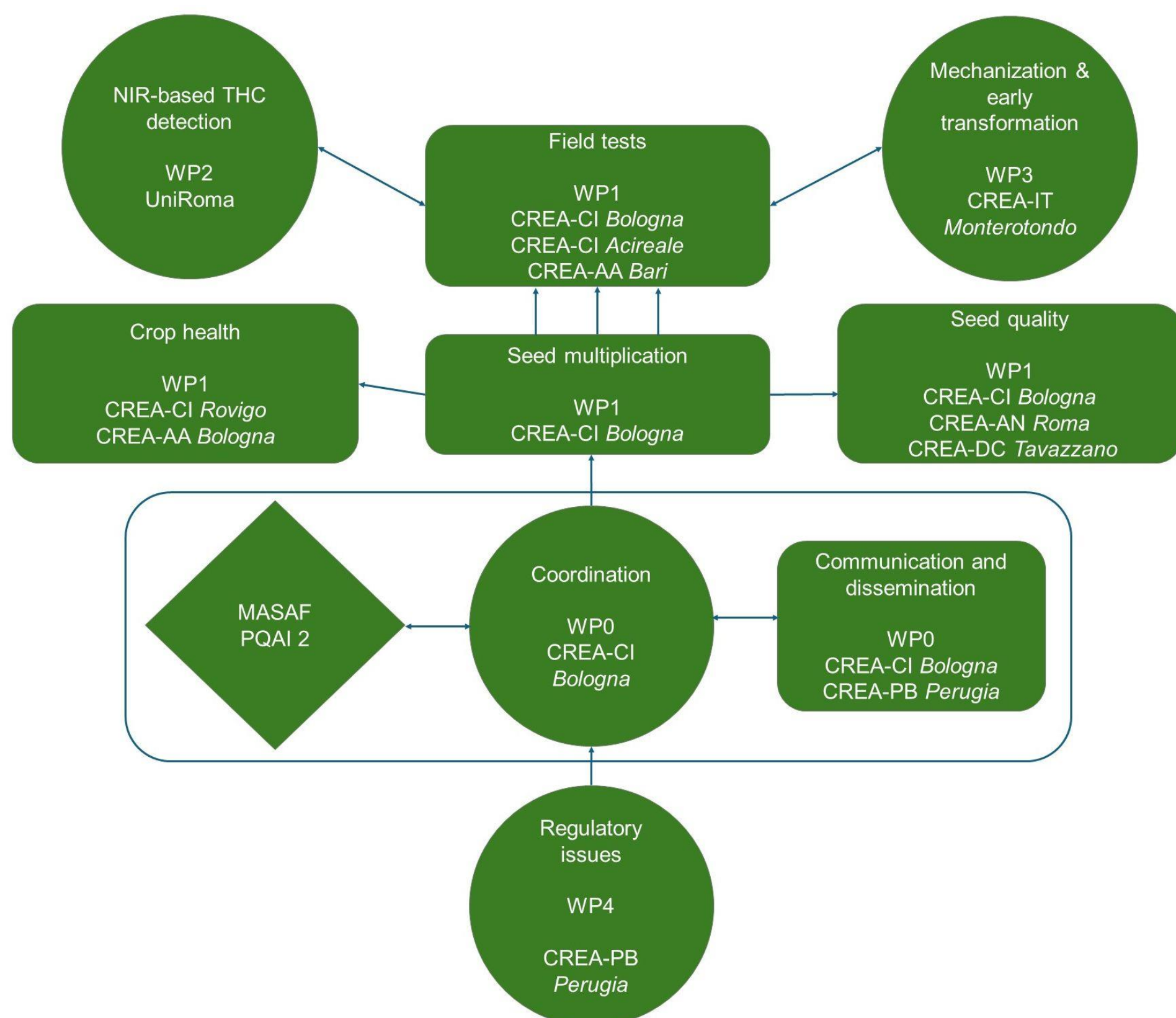
The project is organized into five work packages:

- WP0** – Coordination and dissemination
- WP1** – Genetics and breeding of new seed varieties
- WP2** – Development of NIR-based methods for rapid *in situ* THC analysis
- WP3** – Improvement of mechanization of hemp cultivation and processing
- WP4** – Regulatory issues for the cultivation and seed sector

WP1 is focused on genetics, breeding and biotechnology of industrial hemp

The main research topics are:

- Breeding of new varieties, with special focus on seed yield and quality
- Sequencing of Italian industrial varieties and development of a SNP panel for varietal identification and breeder's rights protection
- Study of diffusion and molecular bases of the presence of THC and other minor cannabinoids in industrial varieties
- Implementation of *in vitro* tools for hemp functional genomics and gene editing
- Development of priming strategies to increase germination vigor and uniformity and study of their molecular basis (see poster 2.17)



Breeding of new hemp varieties

Today's end uses of hemp require a new plant ideotype, with high seed and biomass yields, and suitable for mechanical harvesting. Beyond the maintenance of the old traditional dioecious fibre Italian varieties, new monoecious varieties have been bred at CREA in the frame of CaRiFIT and are on their way to be included in the National and European list of varieties.

In the table are listed the Italian hemp varieties maintained at CREA, or recently developed or under breeding, with the exclusion of the medical ones (see poster 6.31). All these varieties have a THC amount *in planta* < 0.30%, in compliance with EU regulations on industrial hemp cultivation.

Variety	Type	End use	RNV registration	CPVO patent	WGS within CARIFIT project
Carmagnola	Dioecious	Fibre/Biomass	Yes		Yes
CS	Dioecious	Fibre/Biomass	Yes		
Fibranova	Dioecious	Fibre/Biomass	Yes		Yes
Eletta Campana	Dioecious	Fibre/Biomass	Yes		Yes
Fibrante	Dioecious	Fibre/Biomass	Yes	Yes	Yes
Asso	Dioecious	Fibre/Biomass	Yes	Yes	Yes
Codimono	Monoecious	Seed/Biomass	Yes		Yes
Carmaleonte	Monoecious	Seed/Biomass	Yes	Yes	Yes
Felsinea	Dioecious	Fibre/Biomass	approved		Yes
Ermo	Monoecious	Seed	No	Yes	
CARIFIT1p	Monoecious	Seed	under selection		Yes
CARIFIT2t	Monoecious	Seed/Biomass	under selection	Yes	Yes
P2	Dioecious	Ornamental	under selection		

Whole genome sequencing of hemp varieties

In the frame of CaRiFIT, ten Italian industrial varieties will be fully resequenced (see above Table). Illumina WGS will be performed at 50x depth on bulk samples of 20 plants per variety.

The data will generate panels of SNPs and Indels useful for varietal characterization and identification, and for comparison to non-Italian hemp varieties, to highlight the genetic variability existing in a wide pool of industrial hemp germplasm.

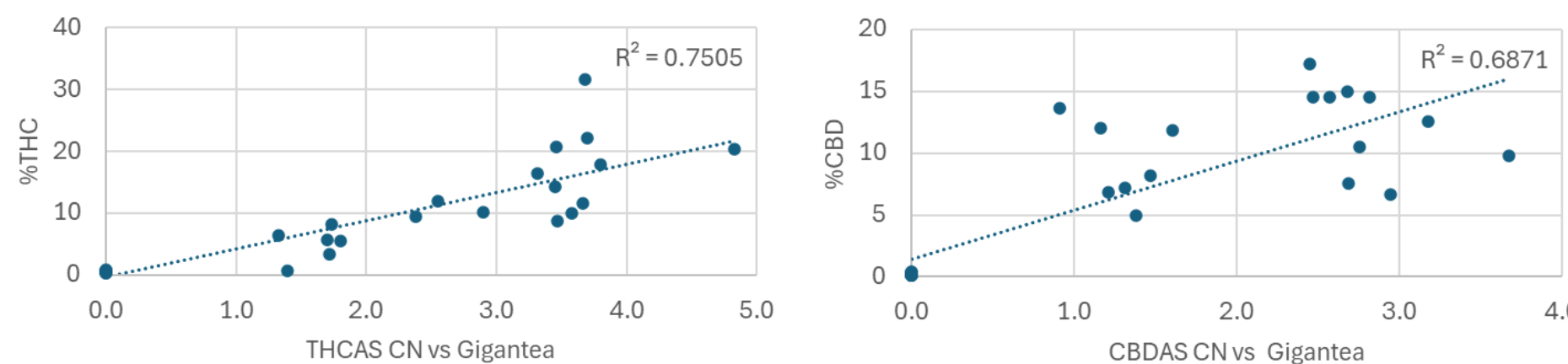
Understanding diffusion and molecular bases of THC in industrial varieties

It was previously found in seed stocks of the oil cv «Finola» that almost 40% of individuals carried an active THCA synthase (doi.org/10.1016/j.gene.2023.147198); similar analyses were performed on the Italian varieties Eletta Campana, Carmagnola and Carmono. No plants carried THCA synthase sequences, but some individuals still had THC levels above 0.2%. Interestingly, some were enriched in CBG, the precursor of CBD and THC.

Variety	% individual with active THCA synthase	% individuals with THC > 0.2%	% individuals with CBG > 0.5%
Finola	38.0	28.6	nd
Carmagnola	0	6.3	8.3
Eletta Campana	0	4.0	2.0
Carmono	0	0	9.1

Ongoing gene sequencing, expression and copy number studies to uncover the genetic basis of the accumulation of residual THC and of CBG aim to obtain:

- Faster and more reliable markers to identify chemotypes of industrial and/or medical relevance
- Target sequences to be used in gene editing, towards new varieties THC-free and/or enriched in specific cannabinoids.



In vitro technologies for gene editing

Establishing a protocol for *in vitro* micropropagation offers a foundation for functional genetics experiments aimed at evaluating the activity of cannabinoid synthase genes, which are still difficult to characterize. In the framework of the CaRiFIT project, CREA - CI Bologna is engaged in the optimization of *in vitro* regeneration protocols and in the use of CRISPR/Cas9 constructs -obtained by Golden Braid-gRNA/Cas9 vectors- for knocking out THCA-, CBDA- and CBCA-synthases.

Preliminary results on regeneration show significant differences according to the type of starting explant (hypocotyl, cotyledon, leaf), substrate composition, and genotype.

