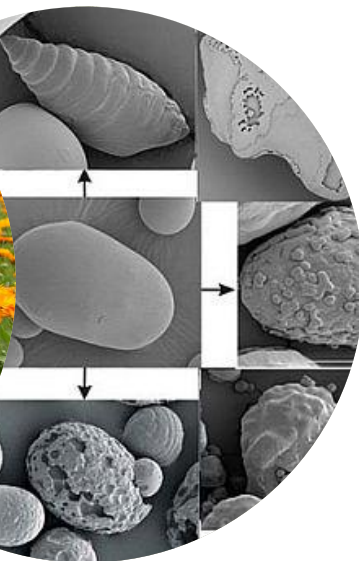


Breeding Hemp varieties for the future

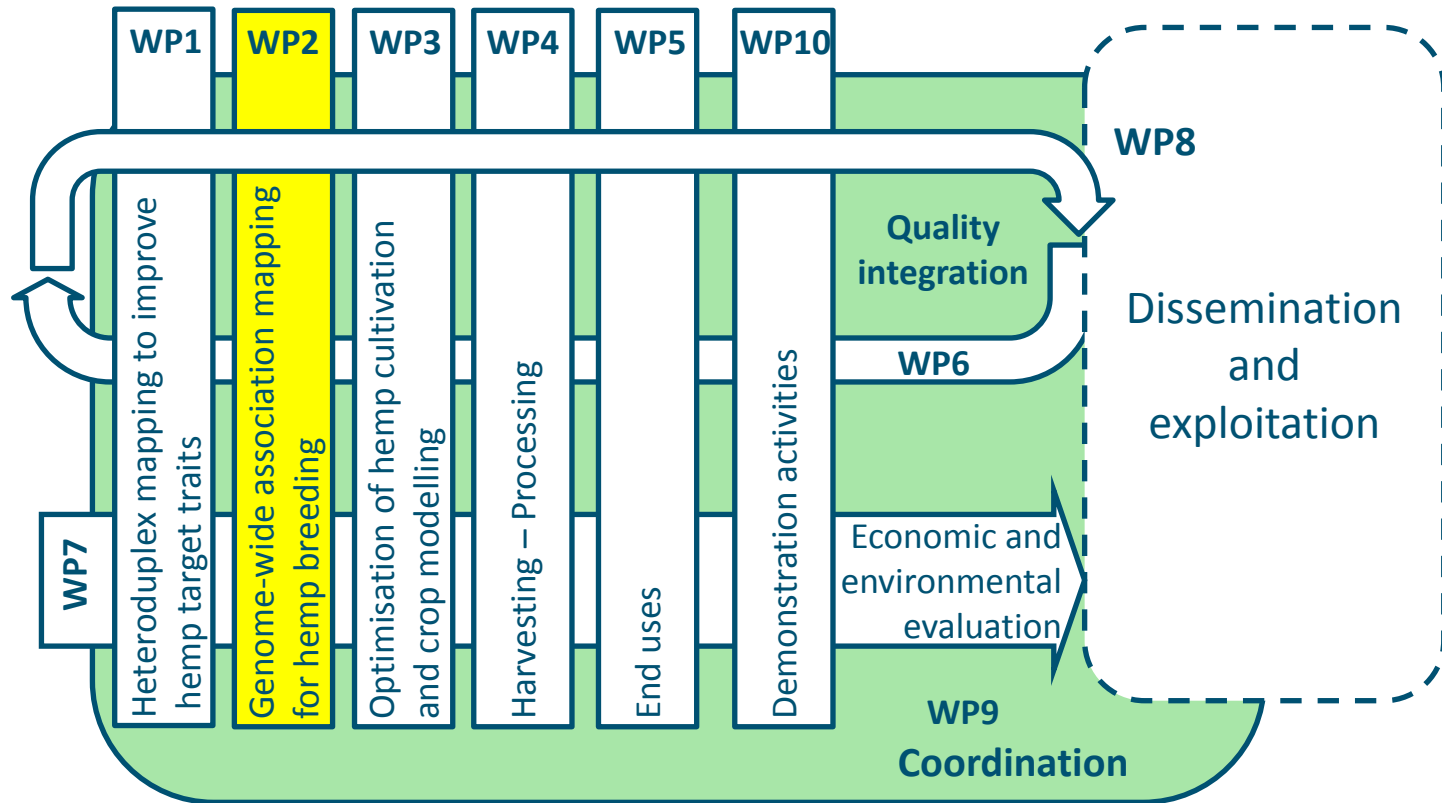
WP2 - Genome-wide association mapping for hemp breeding

Luisa Trindade

Group leader Biobased Economy



MultiHemp



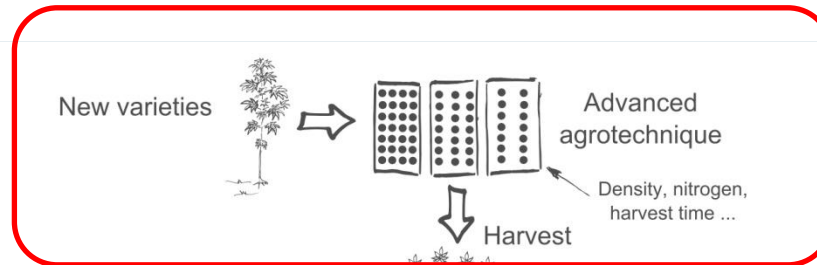
Genome-wide association mapping for hemp breeding - **Work Package 2**

Participants WP2

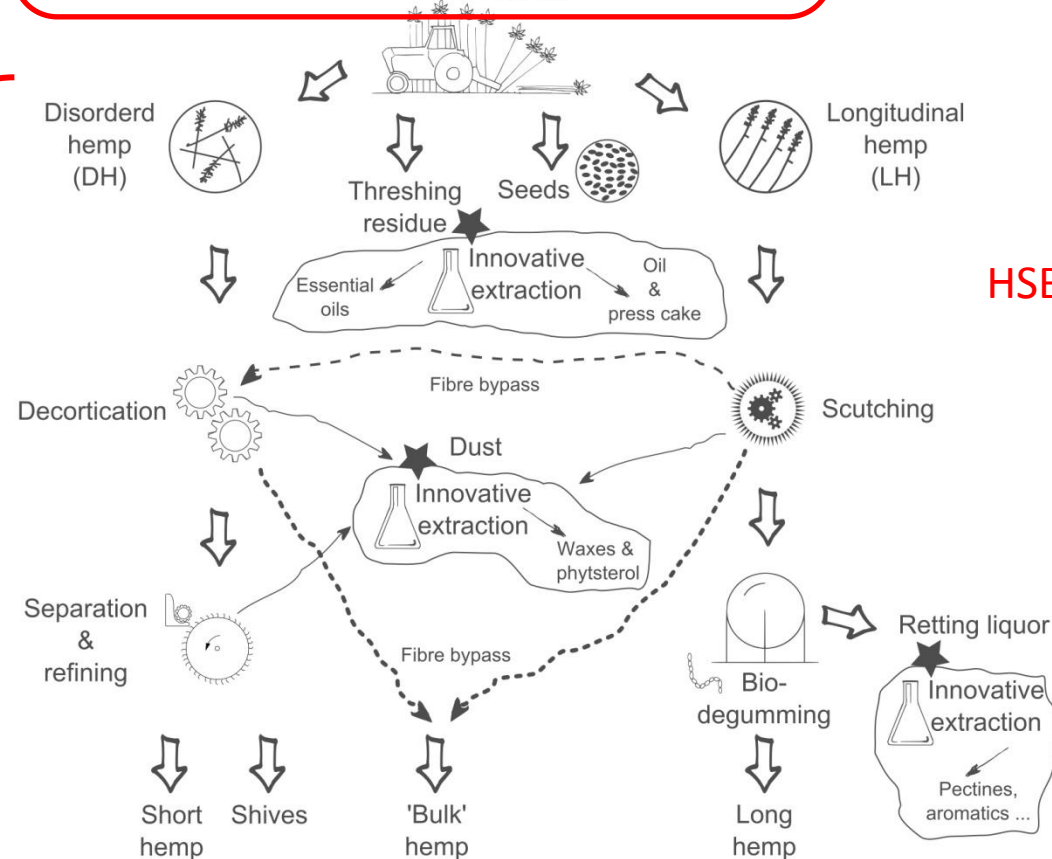
Participants		MM
1 - Università Cattolica del Sacro Cuore	Stefano Amaducci	10
2 - Wageningen University	Luisa Trindade & Elma Salentijn	69
4 - University of Applied Sciences Bremen	Jörg Müssig	34
5 - Leibniz-Institute for Agricultural Engineering	Hans-Jörg Gusovius	22.5
6 - Research Centre for Industrial Crops	Gianpaolo Grassi	40
9 - Federation National Producteurs de Chanvre	Olivier Béhérec & Claire Thouminot	41
10 - VanDijk Semo	Henk van Dijk & Eibert Tichelaar	20
11 - Latgale Agricultural Research Centre	Talis Laizans & Veneranda Stramkale	14
22 - Institute of Bast Fiber Crops	Tang Shouwei, Yang Ming, Zang Gonggu	~~~
		250.5

MultiHemp

Integrated hemp biorefinery



UCSC, WU, CRA,
FNPC, VDS, LARC,
IBFC



HSB, ATB

WP2 -Genome-wide association mapping for hemp breeding

Overall objective

To support the **development of new hemp varieties** optimized for different industrial processes resulting in bioproducts and biomass with an **high added value**

Specific objectives

- Characterization of the **natural genetic** and **phenotypic diversity** in hemp, represented here in a highly diverse collection of **100 hemp accessions**, using a genome-wide association study.
- **Identify genes/QTLs** (or regions in the genome) underlying **morphologic traits**, such as plant height, number and length of the internodes and flowering time.
- Identification of genes associated with the **quality of hemp biomass** such as fibre quality for different industrial applications.
- Integrate this knowledge into existing and new hemp breeding programs to generate **novel hemp varieties** which can more efficiently be converted into biobased products.
- **Develop a set of tools for the breeding of hemp**, both for genotyping and phenotyping.
- Develop a **pipeline for the generation of new hemp varieties** for the different European environments and needs.

MultiHemp WP1 $\neq$ WP2

WP1: Reverse genetics

- Genes  Phenotype
- Mutations
- Candidate gene approach
- Shorter and directed approach

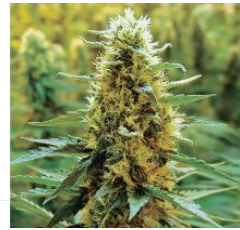
WP2: Forward genetics

- Phenotype  Genes
- Natural diversity
- Based on variability in traits
- Longer approach



Combination of both approaches to speed up hemp breeding

Tools available



van Bakel et al. *Genome Biology* 2011, **12**:R102
<http://genomebiology.com/2011/12/10/R102>



Genome Sequence

RESEARCH

Open Access

The draft genome and transcriptome of *Cannabis sativa*

Harm van Bakel¹, Jake M Stout^{2,3}, Atina G Cote¹, Carling M Tallon³, Andrew G Sharpe³, Timothy R Hughes^{1,4*} and Jonathan E Page^{2,3*}

Genome Browser

Home Genomes Blat Tables PCR Session FAQ Help

C. sativa (*Cannabis*) Genome Browser Gateway

The UCSC Genome Browser was created by the [Genome Bioinformatics Group of UC Santa Cruz](#).
Software Copyright (c) The Regents of the University of California. All rights reserved.

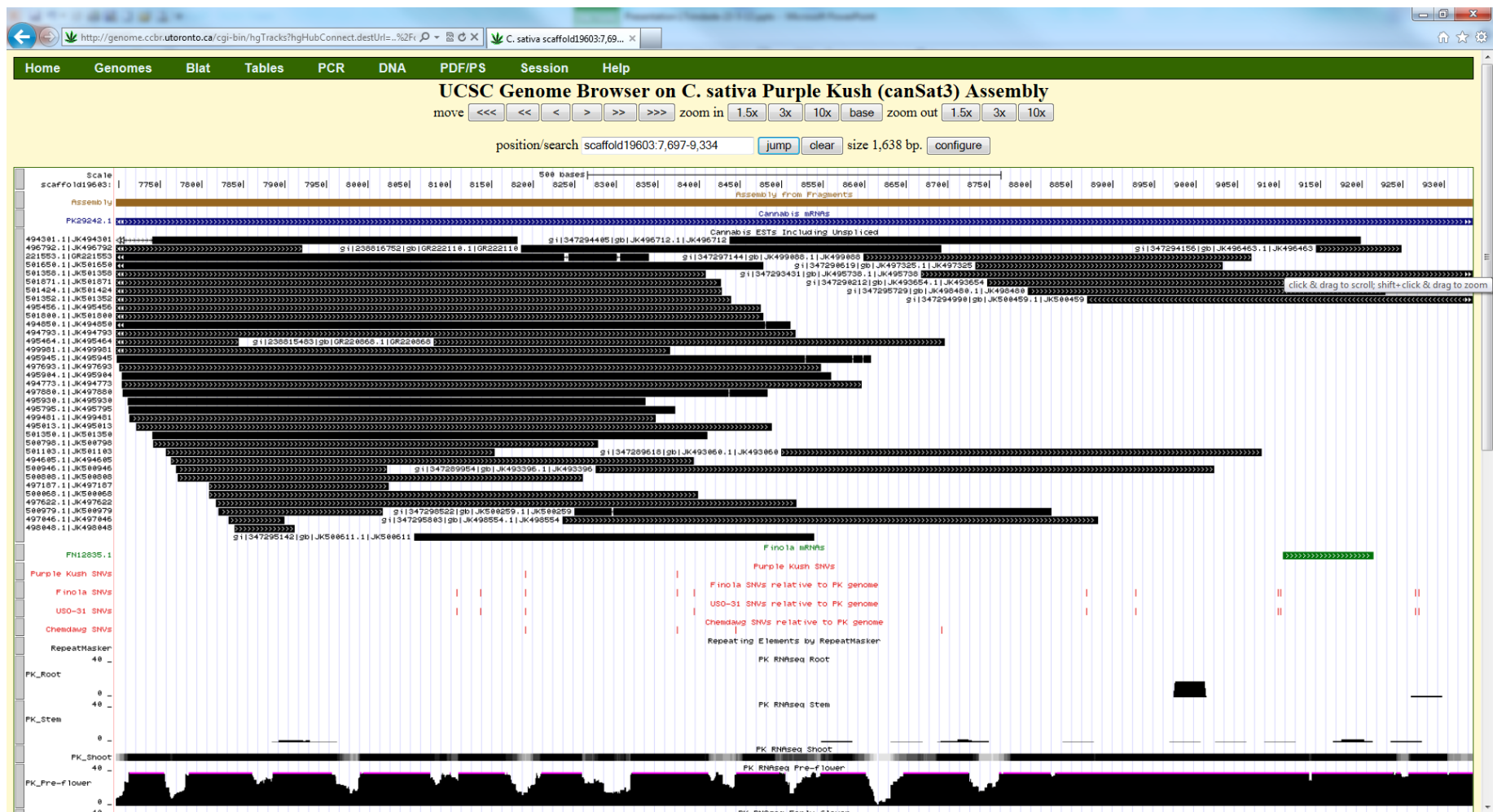
clade	genome	assembly	position or search term	
Plant ▼	C. sativa ▼	Purple Kush (canSat3) ▼	scaffold19603:7,697-9,334	submit

[Click here to reset](#) the browser user interface settings to their defaults.

Assembly overview

Minimum scaffold size:	400 bp
Total bases (+gaps)	787.3 Mb
Total bases (-gaps)	534.7 Mb
N50	16.2 kb
Maximum scaffold size:	565.9 kb
Number of scaffolds:	136,377

Genome Browser



Genome-wide association mapping for hemp breeding

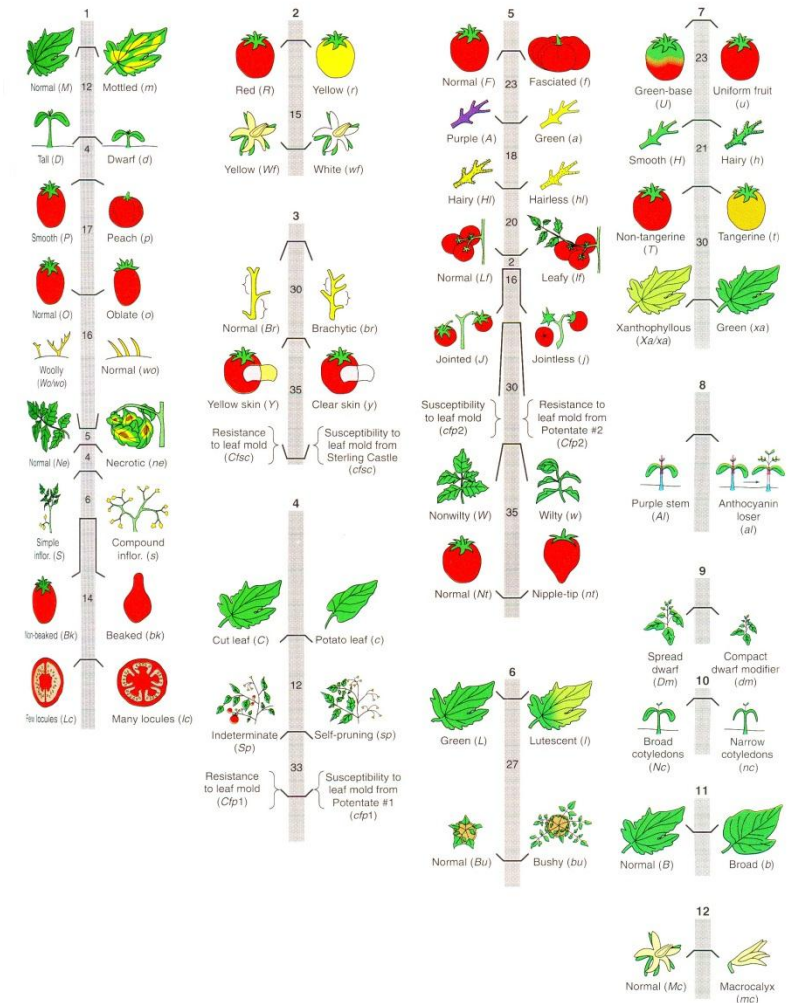
Association mapping

Def: Association between different traits (e.g. yield, fibre quality, oil composition) and polymorphisms in the genome.

Basis: Natural genetic variation, and linkage disequilibrium (non-random association of alleles)

Advantage: it can map quantitative traits with high resolution in a way that is statistically very powerful.

Applications: very widely applied in species in all kingdoms, where natural variation exists.



Genome-wide association mapping - GWAS

Number of associations
identified for dif. P-values for
each *A. thaliana* genotype

Approach:

1) Sequencing the genome of
100 hemp accessions.



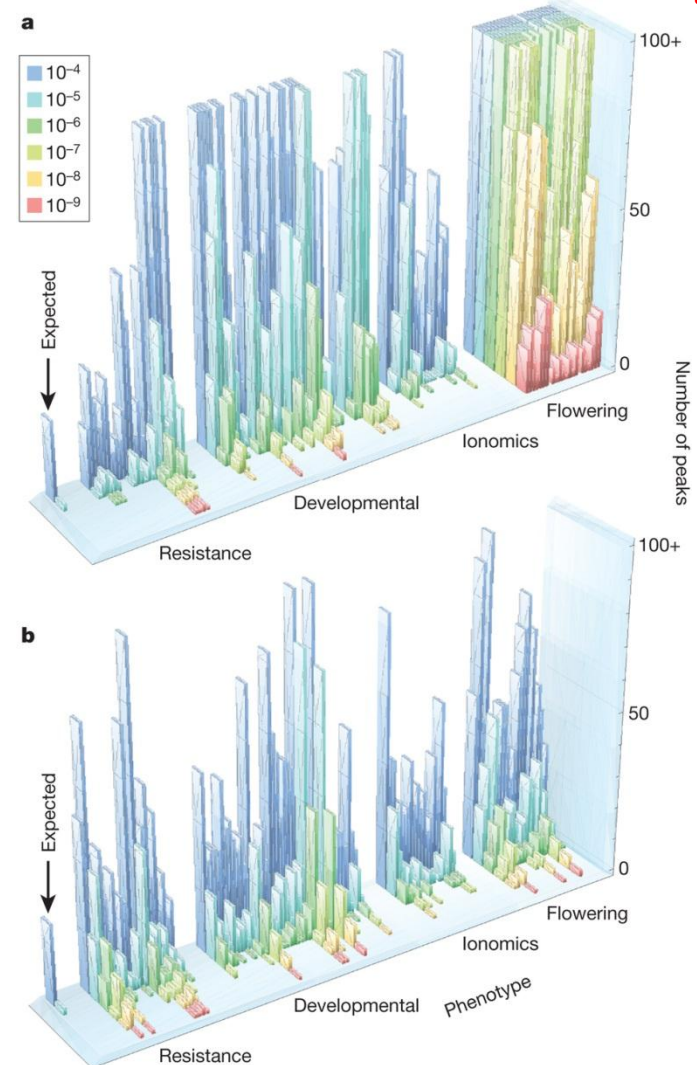
Massive sequencing

2) Phenotype different relevant
traits in the 100 hemp accessions



Fibre length, fibre composition,
etc.

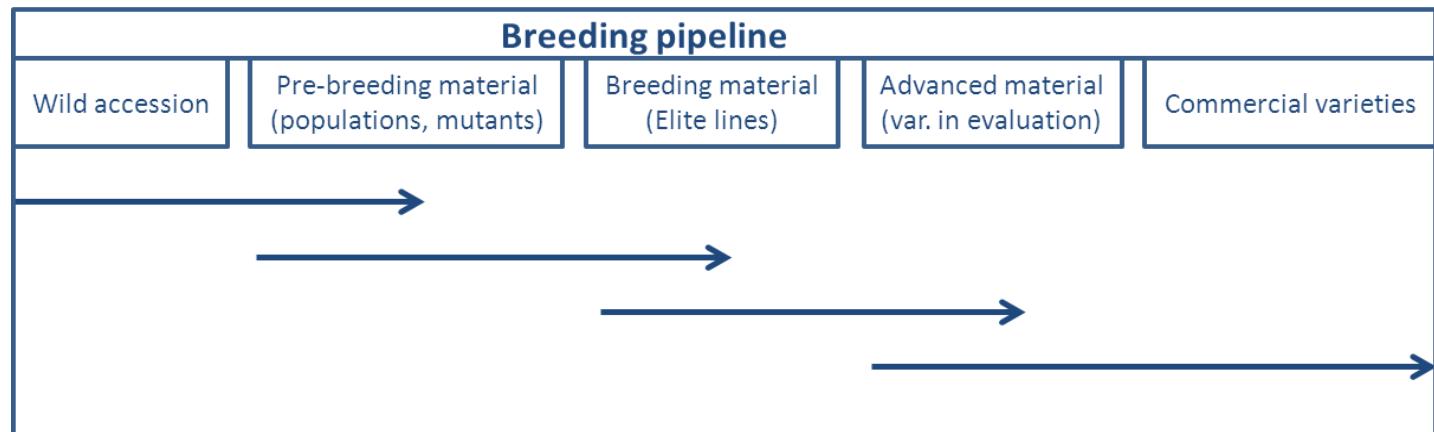
3) Find a linkage between traits
and alleles



Atwell et al., 2010. *Nature*. 465: 627-31

Integrate the knowledge in hemp breeding programs

Development of new varieties is a long path (up to >20 years)



Integration of the knowledge in different steps of breeding programs

How far are we?

Tasks (till 2017)

Task 2.1 Seed propagation of association mapping (AM) panel (WU, VDS, FNPC, CRA, IBFC) **DONE**

Task 2.2 Genotyping (WU, VDS, IBFC) **In prep.**

Task 2.3 Phenotyping (UCSC, WU, UoY, VDS, HSB, FNPC, CRA, LARC, IBFC) **In prep.**

Task 2.4 Association mapping (WU)

Task 2.5 Improve fibre quality in elite cultivars (VDS, FNPC, LARC and CRA)

Task 2.6 Breeding pipeline (WU, VDS, FNPC, LARC, CRA)

Deliverables

D 2.1 Seeds for the 100 hemp lines (month 12) **DONE**

D 2.2 Deep sequence data of 100 hemp lines (month 30)

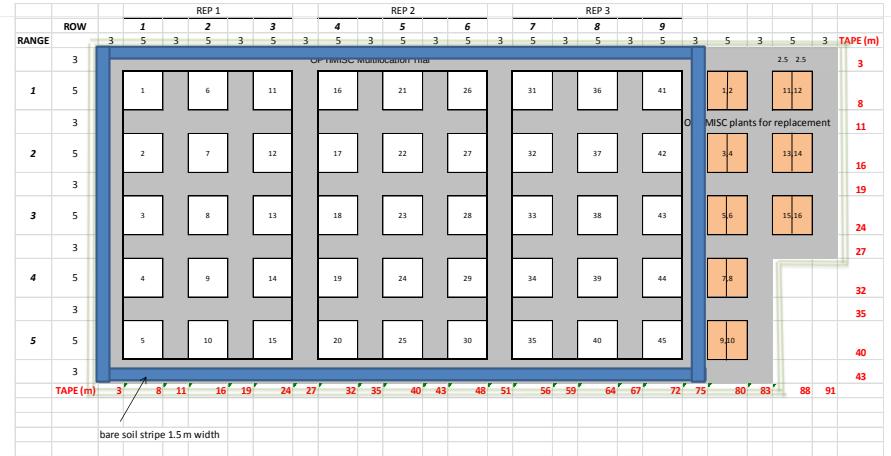
D 2.3 Physical map containing SNPs identified in the AM panel (month 36)

D 2.4 Genes underpinning relevant quality traits identified (month 48)

D 2.5 Fibre quality traits integrated in Elite lines (month 54)

126 hemp lines in 3 reps

Randomized blocks



126 hemp lines in 3 locations: Italy, Netherlands and France



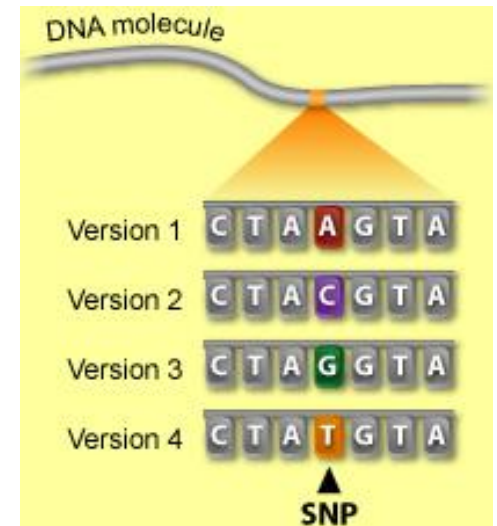
100 lines for AM

Phenotyping of the 100 hemp accessions

- **Physiological traits**
 - Emergence rate
 - Flowering time
 - Number and length of internodes
- **Quality traits**
 - Fibre quality
 - Cell wall composition
 - Fibre length
 - Yield
 - THC content

Genotyping of 100 hemp lines

- **For each of the 100 lines**
 - Collect 1 representative plant + 9 plants
 - Illumina massive sequencing
 - Identification of Single Nucleotide Polymorphisms (SNPs)
 - Correlate SNPs with different traits



Follow up steps in WP2

- Finalise the protocol for phenotyping of the AM field trial
- Phenotyping of the field trials in 3 locations: F, I and NL
- Collect plant material for genotyping of the 100 accession by massive sequencing

Thank you!



Questions?