Research at SLU Targeting Industrial Quality Oils in Traditional and New Oil Crops

Prof. Anders S. Carlsson/ Head of Department of Plant Breeding

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Overview

- Department of Plant Breeding, SLU
- Crambe abyssinica as a platform for production of tailor made industrial oils
- Lepidium campestre (Field cress) as a new under-sown oil crop
- GM camelina producing an oil that contains sex pheromones for specific insect pests
- Identifying the underlying mechanisms for oil composition in Rapeseed
- Precision breeding in Rapeseed
Department of Plant Breeding at SLU

Ca. 75 colleagues
(incl. about. 25 PhD students (many on fellowships)
15 technical personnel
30 junior & senior researchers)

Head of Department: Prof. Anders S. Carlsson anders.carlsson@slu.se
Deputy Head: Dr. Mariette Andersson mariette.andersson@slu.se

Five divisions with their subject area

- **Agricultural Plant breeding**
  Lead by Prof. Rodomiro Ortiz
  rodomiro.ortiz@slu.se

- **Horticultural Plant breeding**
  Lead by Dr. Kimmo Rumpunen
  Kimmo.rumpunen@slu.se

- **Agricultural plant quality**
  Lead by Prof. Eva Johansson
  Eva.johansson@slu.se

- **Horticultural plant quality**
  Lead by Prof. Marie Olsson
  Marie.olsson@slu.se

- **Plant biotechnology**
  Lead by Assoc. Prof. Per Hofvander
  Per.Hofvander@slu.se

Plant properties:

- Pre Harvest
- Post Harvest
Developing a new oil crop by domestication of field cress (Lepidium campestre) using conventional and biotechnology approaches

Contacts for more information:
Mulatu.Geleta.Dida@slu.se
Li-Hua.Zhu@slu.se
Domestication of field cress (Lepidium campestrum)

- It shares the Brassicaceae family with *Brassica* & *Arabidopsis*
Field cress (Lepidium campestre)

- 2n=2x=16
- biennial
- winter hardy
- potential for high seed yield
- industrial quality oil

- suitable as under-sown crop
Undersown field cress

Undersowing field cress with spring cereals

- It serves as cover/catch crop during the first year (autumn/winter)
- and harvested as an oil crop the second year

- In a separate line we are developing a perennial type that can provide better ecosystem services
  - higher carbon storage
  - reduced input costs
  - better energy use efficiency
  - better soil and water management
Major traits targeted

- Pod shattering
- Seed yield
- Oil content
- Synchronous maturity
- Seed dormancy
- Perenniality
- Pathogen resistance
Developing genomic tools and resources

**Approaches used**
- RAD-Sequencing
  - For marker development
- Genotyping by Sequencing
- Whole Genome Sequencing
  - (NextSeq used)

**Genome size**
- **533 Mbp**
  - Used as reference genome for GBS based SNP discovery

**Assembled sequence size**
- **219 Mbp**

**Institute of Biotechnology, Cornell University, USA**

**Edinburgh Genomics, UK**

- To speed-up the domestication process
  - Efficient use of genetic resources
  - Marker-assisted selection
Major traits targeted for breeding:
- Pod shattering, Oil content, synchronous maturity, seed yield and perenniality

• Pod shattering:
  - Pod-shatter resistant Breeding lines that lose less than 10% of their seeds conditions that wild type genotypes loose more than 90% of their seeds are developed. The resistant lines as good as rapeseed

• Oil content:
  - In some breeding lines, oil content has been increased by up to 35% (from ca 20% to ca 27%)

• Synchronous maturity:
  - Few perfect synchronously maturing breeding lines have been developed

• Seed yield:
  - During yield trial in 2015/2016, up to 4.5 t/ha per breeding line under insufficient rain fall condition

• Perenniality
  - Perennial breeding lines have been developed through interspecific hybridization between Lepidium campestre and L. heterophyllum
Different approaches have been used to develop genomic tools and resources

- **RAD-Sequencing produced:**
  - More than 190,000 DNA sequences (117-567 bp)
    - used to develop 1700 microsatellite markers
  - About 90,000 SNPs
  - 9K SNP chip developed and used to genotype 1200 samples
    - For GWAS and QTL mapping

- **Genotyping by Sequencing (GBS) produced**
  - About 126,000 SNPs
  - QTL mapping underway

- **Whole genome sequencing**
  - Genome size = 533 Mbp

- **Genetic linkage map developed**
  - contains 10,302 contigs and 1044 SNPs

- 30 genes coding for desirable traits were identified through comparative genomic analysis of Lepidium and Arabidopsis genomes
Genetic improvement of *Lepidium campestre* using biotechnology approaches

Results achieved using GM approach

• **High oleic acid:**
  • increased from 11% in WT to 80% in GM-line

• **High oil content:**
  • increased from 20% in WT to 27% in GM-line by overexpression a transcription factor gene and hemoglobin genes.

• **Reducing pod shatter:**
  • GM-lines with reduced pod shatter are obtained by down-regulating one key gene regulating the pod shatter

• **Wax ester production:**
  • over 25% wax esters were produced in lepidium seeds for industrial applications by overexpressing the jojoba wax synthesis genes

Current research focus

• **Trait stacking:** combine improved traits into single improved lines
• **Using the CRISPR technique to obtain transgene free improved lines**

*Ivarson et al. BMC Plant Biol., 2013*

Mistra Biotech supported by Mistra and SLU
Developing Crambe abyssinica as a platform for production of tailor made industrial oils.

Contacts for more information:
Li-Hua.Zhu@slu.se
Per.hofvander@slu.se
Anders.carlsson@slu.se
Ida.lager@slu.se
Crambe abyssinica: industrial crop

Crambe fulfills the criteria for a robust and safe oil crop platform for industrial products:

- Not a food crop (60% erucic acid)
- No outcross with other oil crops or wild relatives in northern Europe
- Reasonable oil yield per hectare (same as spring rape)
- Already in production as an agricultural crop
- Excellent fatty acid composition of oil for the conversion into wax esters and ultra-high erucic acid content

Financial sources: EU-ICON, FORMAS and SSF
Achievements so far with crambe

- **Extra high erucic acid (HEA):** increased from 60% in WT to 73% in GM-line by regulating three key genes involved in fatty acid biosynthesis.

- **Wax ester production:** up to 50% of wax esters in the seed oil in GM-lines compared to zero in WT by overexpressing 3 jojoba wax synthesis genes.

- **High oleic acid:** increased from 15% in WT to 80% in GM-line by regulating 2 key genes involved in the seed oil biosynthesis.

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*Guan et al. Plant Biotech J., 2013*

Field trials on HEA and wax GM lines

The results showed reduced seed setting and seed oil for both types of GM lines. For the wax lines, reduced germination.
Ongoing research

Studies on molecular mechanisms limiting further increase in erucic acid level and investigating the mechanisms underlying the altered agronomic traits in the different GM-lines through functional analysis of some important genes involved in oil biosynthesis

- DGAT (Specificities of DGATs partly determine the oil quality)

![Two isoforms of DGAT](image)

- Transcriptome analysis for wax ester synthesis in transgenic crambe, etc.
Production of plant oils containing sex pheromones for targeting insect pests

Contacts for more information:
Per.hofvander@slu.se
Plant oils containing pheromone components for use in insect pests traps

Genetically modified camelina produce an oil with specific fatty acids. After extraction, these can be modified into unique aldehydes, acetates and alcohols, which are active as sex pheromones for specific insect pests.
Camelina sativa (Gold-of-pleasure) with oil containing pheromone precursors

May, 2016—Nebraska

Oil composition CpuFatB-AtrD11

After seed oil extraction, fatty acids are isolated and chemically modified into active pheromone compounds that are used to prepare pest insect traps for field testing.
Field trapping experiment 2017

Time: early March, 2017
Place: Canton, China
Plants: cauliflower, radish et al

Treatments:
- primarily purified product (precursor purity 38%)
- highly purified product (precursor purity 83%)
- synthetic pheromone (optimal)
- negative control (solvent alone)

Results from field trapping experiment

Choy sum, Brassica rapa var. parachinensis

22° 8' N, 113
Identifying the underlying mechanisms for oil composition in rapeseed

Contacts for more information:
ida.lager@slu.se

In collaboration with:
Identifying the underlying mechanisms for oil composition in rapeseed

- Studying the role of enzymes that are crucial for regulating the composition of seed oil in rapeseed
- Comparing high erucic and low erucic rapeseed
- Identify the genetic background of importance for the mechanism regulating oil quality
- Knowledge achieved from our research on the seed oil metabolism in Crambe abyssincia provide clues in understanding the mechanism regulating seed oil quality in rapeseed
Precision breeding of rapeseed

Contacts for more information:
Li-Hua.Zhu@slu.se
Precision breeding of rapeseed

- Using CRISPR/Cas technique to produce transgene free
- Target traits: disease resistance, seed yield, herbicide resistance in the first step
- Protoplast approach is used for gene editing by CRISPR and the protoplast approach looks very promising so far as high quality protoplasts can be obtained from rapeseed.
- The project has just started

*Project supported by FORMAS
Lantmännens provides the initial working material

Source: Li-Hua Zhu, SLU, Alnarp