

# Molecular breeding of new seed oil varieties of Industrial Hemp



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# The starting material: Finola® variety:

- non-drug (<0.2% THC), seed oil variety/  
legislative approval
- early flowering (at 25-30 days) and
- early maturing (typically within 115  
days)
- dwarf variety allows use of conventional  
equipment for harvest
- diploid
- can be grown at high latitudes (62°)
- good rotation crop
- low-input crop (sprays and fertilizer)
- seed yield: 1 - 2t/ha



Hemp harvest



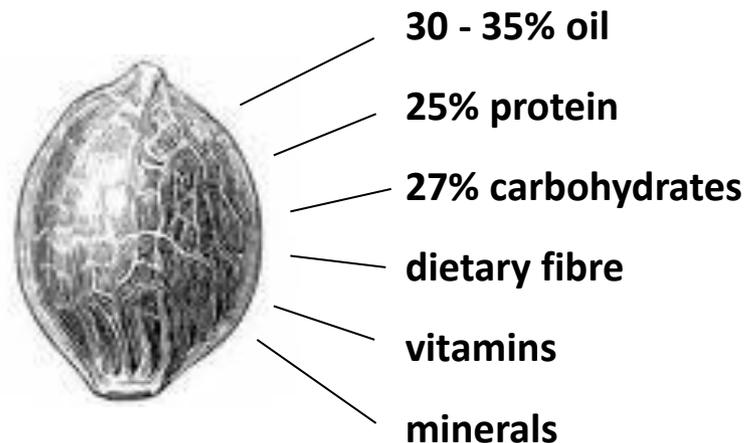
Seed from combine



# *Cannabis sativa* - general features and characteristics



- annual herbaceous plant
- both monoecious and dioecious varieties grown commercially
- grows 3 -10 feet tall
- drug (marijuana) or fibre/oil types (industrial hemp) based on low THC content
- originally native to the Caucasus region of far eastern Europe, northern India, and Persia (Iran)
- now cultivated in warm-to-temperate regions all over the world
- claimed to be suitable for low-input farming

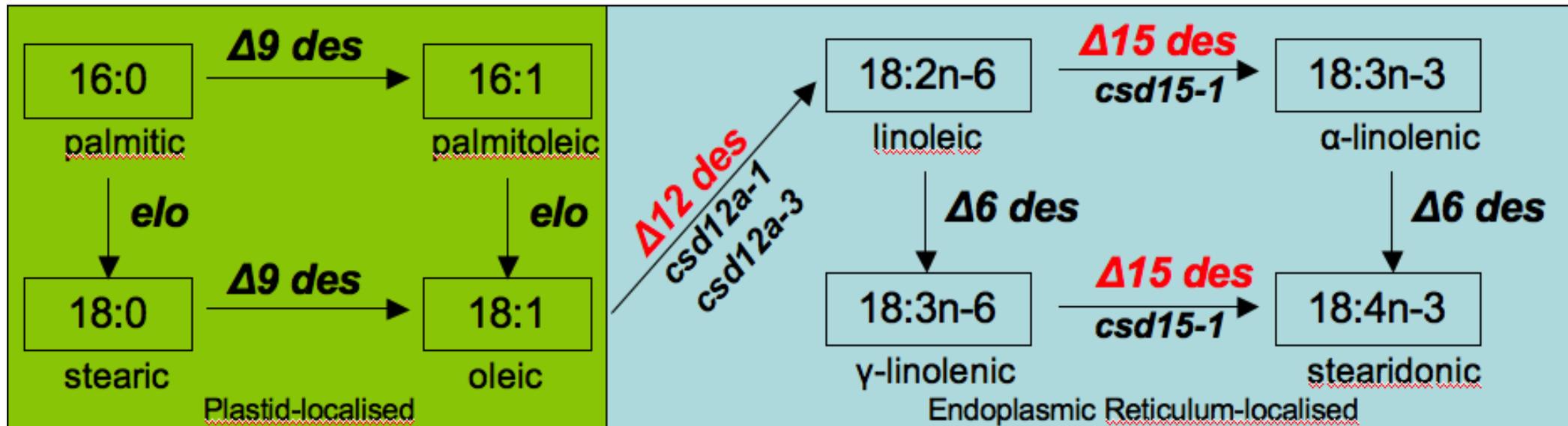


# Typical fatty acid composition (%) of hemp and other vegetable oils

|                  | Palmitic acid<br>16:0 | Stearic acid<br>18:0 | Oleic acid<br>18:1 | Linoleic acid<br>18:2n6 | $\alpha$ -Linolenic acid<br>18:3n3 | $\gamma$ -Linolenic acid<br>18:3n6 | Stearidonic acid 18:4n3 | %PUFA |
|------------------|-----------------------|----------------------|--------------------|-------------------------|------------------------------------|------------------------------------|-------------------------|-------|
| Hemp (Finola®)   | 5                     | 2                    | 9                  | 56                      | 22                                 | 4                                  | 2                       | 84    |
| Flax (linseed)   | 6                     | 3                    | 15                 | 15                      | 61                                 | 0                                  | 0                       | 76    |
| Olive            | 15                    | 0                    | 76                 | 8                       | <1                                 | 0                                  | 0                       | 8     |
| Sunflower        | 5                     | 11                   | 22                 | 63                      | <1                                 | 0                                  | 0                       | 63    |
| Wheat germ       | 3                     | 17                   | 24                 | 46                      | 5                                  | 5                                  | <1                      | 56    |
| Rape seed        | 4                     | <1                   | 60                 | 23                      | 13                                 | 0                                  | 0                       | 36    |
| Soy              | 10                    | 4                    | 23                 | 55                      | 8                                  | 0                                  | 0                       | 63    |
| Evening primrose | 6                     | 1                    | 8                  | 76                      | 0                                  | 9                                  | 0                       | 85    |
| Black currant    | 7                     | 1                    | 11                 | 48                      | 13                                 | 17                                 | 3                       | 81    |
| Borage           | 12                    | 5                    | 17                 | 42                      | 0                                  | 24                                 | 0                       | 66    |

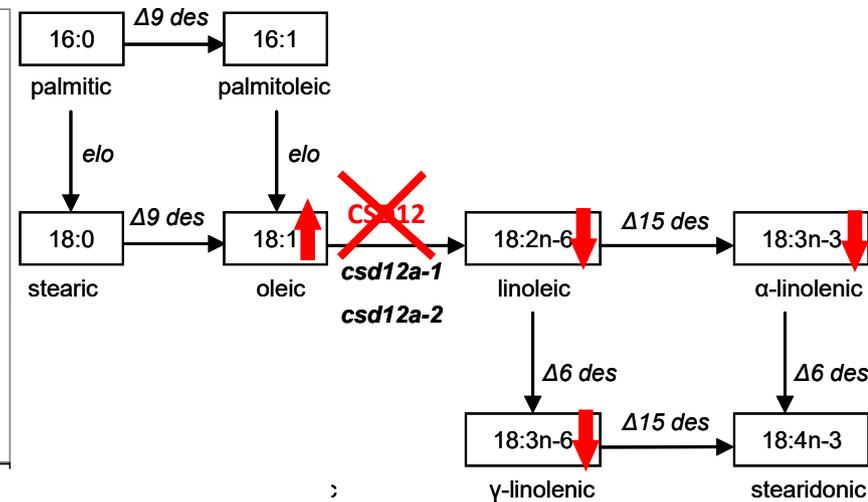
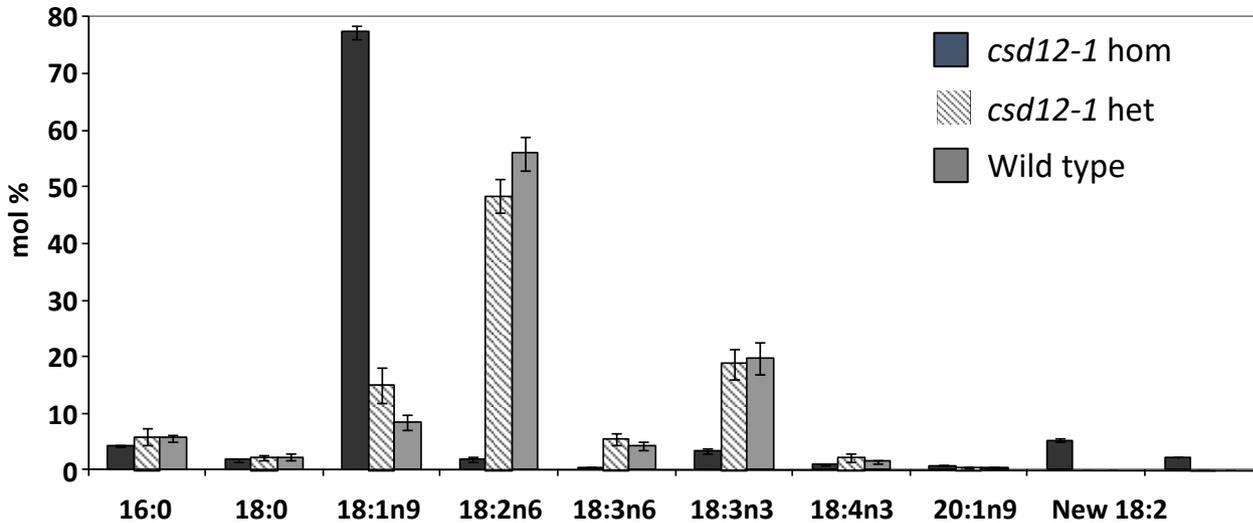
Table modified from Callaway, Euphytica 140:65-72 (2004)

## Summary of PUFA synthesis in hemp seed



# *cds12a-1* and *cds12a-2* are functional knockout alleles of $\Delta 12$ desaturase resulting in high-oleic acid phenotype

## Fatty acid composition of the seed lipids from seeds segregating allele *cds12a-1*



- *cds12a-1* (Q167\*)



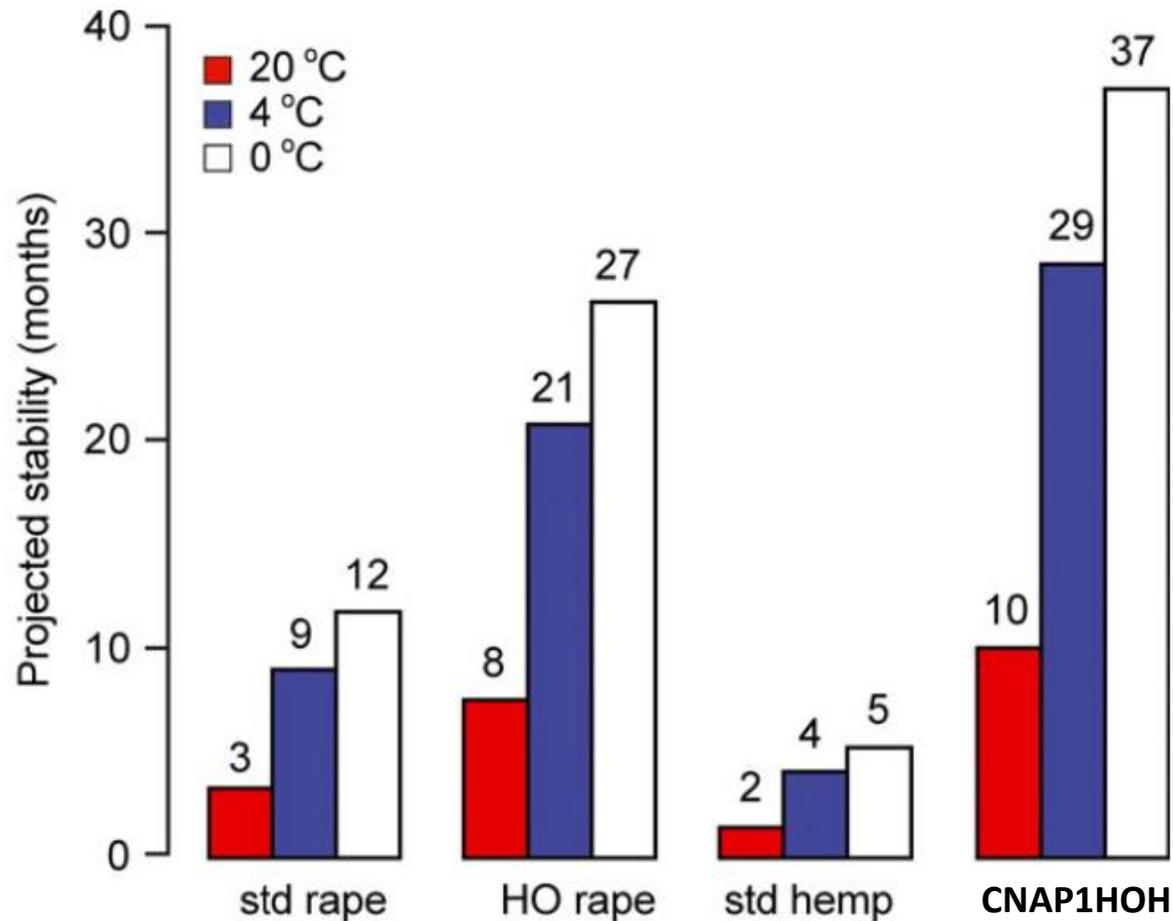
- *cds12a-2* (P218L/P375L)



- high levels of oleic acid (9%  $\longrightarrow$  77%) at expense of PUFAs (>80%  $\longrightarrow$  14%)

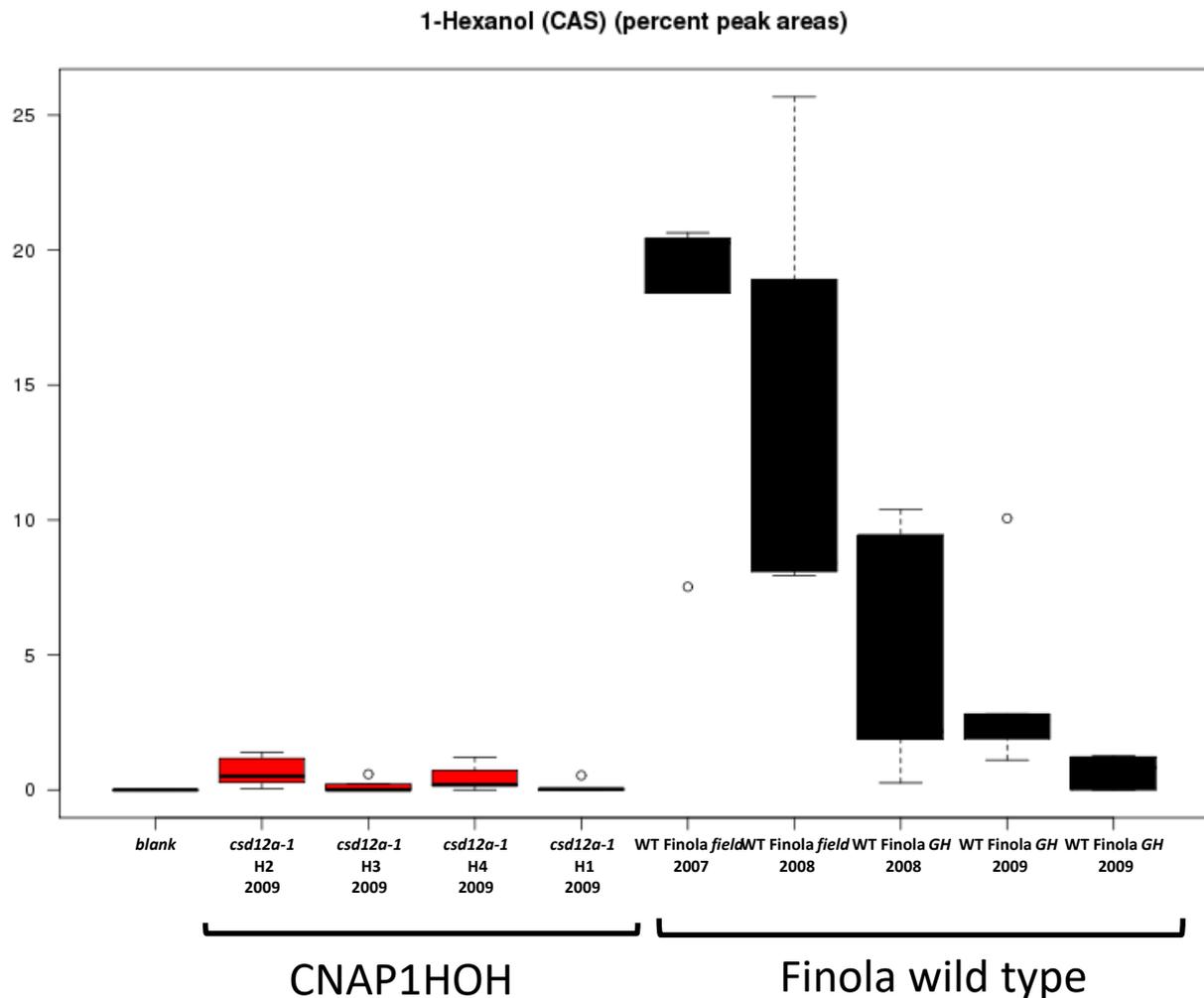
- *cds12a-1* mutant allele was used to develop high-oleic acid hemp variety 'CNAP1HOH'

# CNAP1HOH seed oil has much higher oxidative stability than standard hemp seed oil



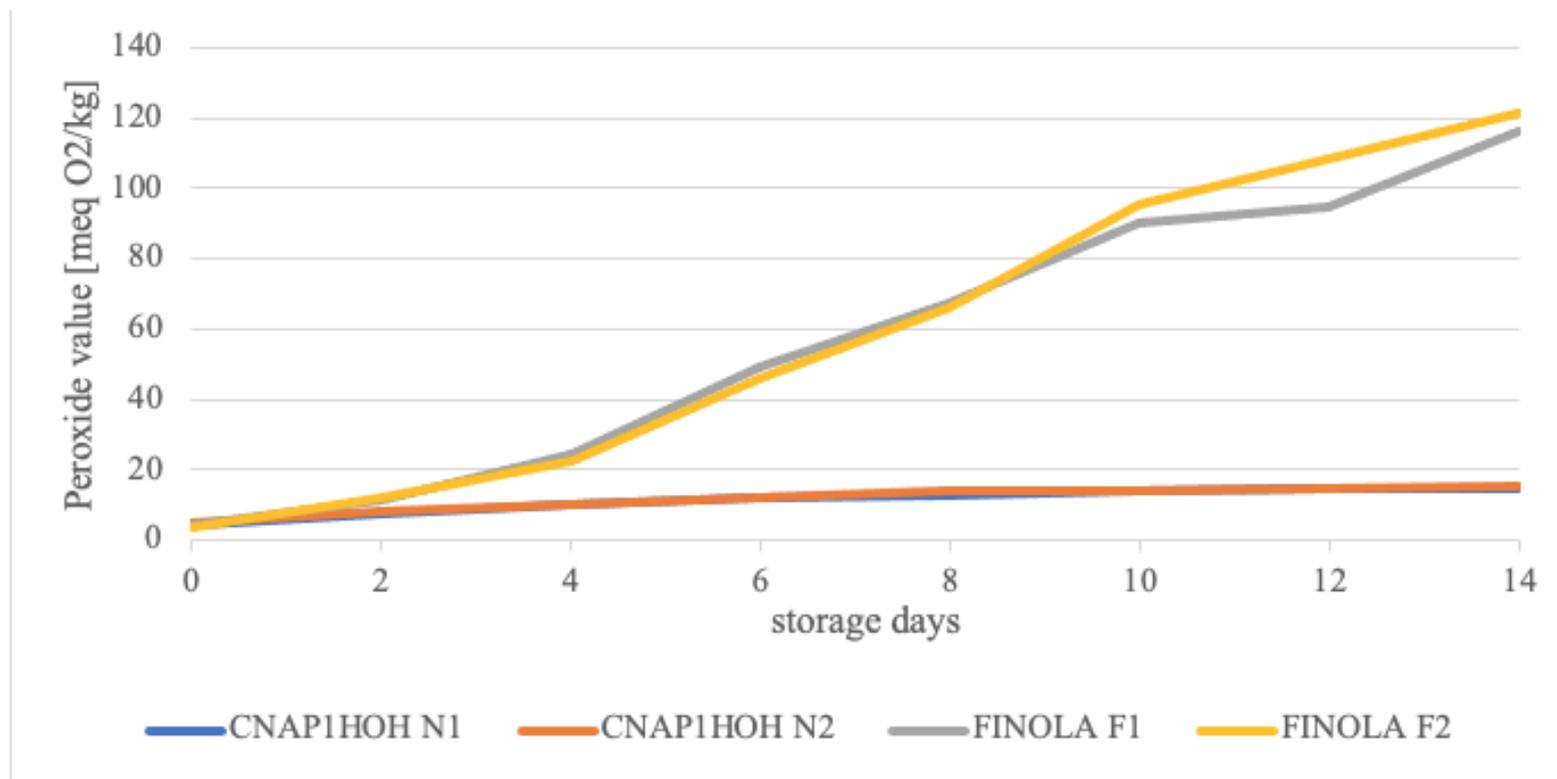
**Cargill High Oleic Canola Oil** 'This stability ensures the performance food manufacturers and foodservice providers need, while delivering the high quality taste and nutritional benefits consumers demand'.

# CNAP1HOH - further characteristics: Volatiles emitted by seed crushing - example Hexanol (by headspace SPME-GCMS)



- medium chain alcohols typical degradation products of PUFAs
- decreased levels emitted by seed crushing indicating lower oxidation/higher stability of high oleic acid oil/low PUFA oil

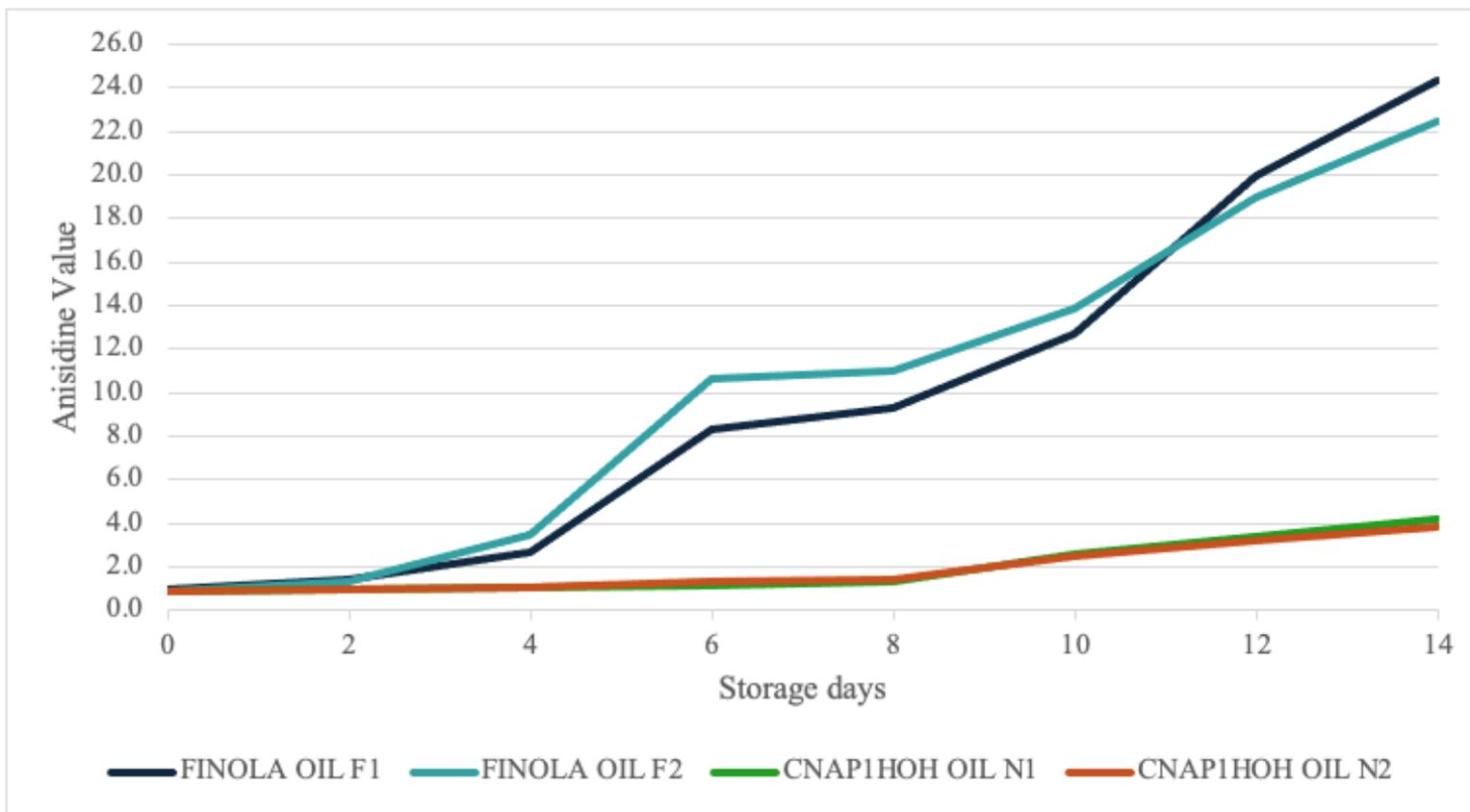
## Independent thermostat test confirms significantly higher oxidative stability of cold-pressed CNAP1HOH hemp seed oil compared to Finola



**Figure 2.2.** Changes in the peroxide value of cold-pressed oils from hemp seeds of the FINOLA (F1 and F2) and CNAP1HOH (N1 and N2) varieties.

Peroxide value indicates the amount of primary oxidation products,

# Independent thermostat test confirms significantly higher oxidative stability of cold-pressed CNAP1HOH hemp seed oil compared to Finola



**Fig. 2.3.** Changes in the Anisidine value of cold-pressed oils from hemp seeds of the FINOLA (F1 and F2) and CNAP1HOH (N1 and N2) varieties.

**Anisidine value indicates the amount of secondary oxidation products**

## **CNAP1HOH – a novel High Oleic Acid hemp seed oil variety with increased stability**

- UK Plant Breeders Rights (plant variety rights) were granted to the University of York in 2021
- Following multiple field trials CNAP1HOH was added to the List of Approved Cultivars in Canada in 2023
- USA and Canadian Patent protection has been granted on the csfad2a-1 mutant allele that results in high oleic acid in the seed oil

# Canadian hemp cultivation 2023

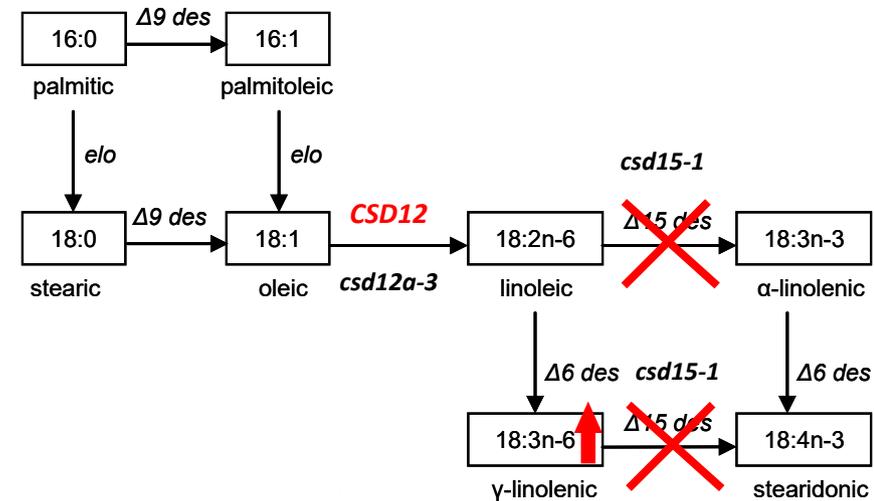
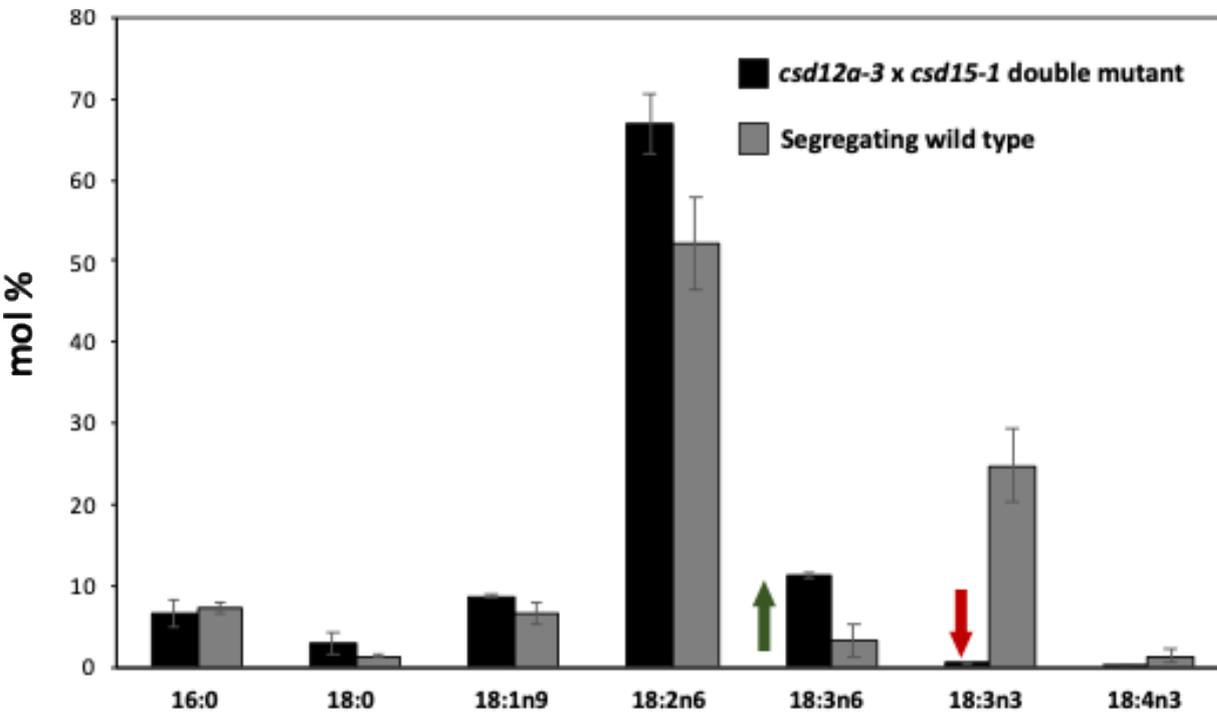
**Finola the most widely grown variety – 40% of total in 2023  
(Data from Health Canada).**

**Table 2: Hectarage for Cultivation of Industrial Hemp by Cultivar by Province**

| Cultivar        | Hectares       |              |                |             |             |               |             |             |               |              |               |                |             | TOTAL           |
|-----------------|----------------|--------------|----------------|-------------|-------------|---------------|-------------|-------------|---------------|--------------|---------------|----------------|-------------|-----------------|
|                 | AB             | BC           | MB             | NB          | NL          | NS            | NT          | NU          | ON            | PEI          | QC            | SK             | YT          |                 |
| <b>Finola</b>   | 2913.34        | 0.00         | 655.18         | 0.00        | 0.00        | 0.00          | 0.00        | 0.00        | 0.00          | 0.00         | 0.00          | 1123.05        | 0.00        | <b>4691.57</b>  |
| <b>CNAP1HOH</b> | 0.00           | 0.00         | 24.69          | 0.00        | 0.00        | 0.00          | 0.00        | 0.00        | 0.00          | 0.00         | 0.00          | 0.00           | 0.00        | <b>24.69</b>    |
| <b>TOTAL</b>    | <b>5595.70</b> | <b>60.35</b> | <b>2770.42</b> | <b>2.70</b> | <b>0.00</b> | <b>133.55</b> | <b>0.00</b> | <b>0.00</b> | <b>124.25</b> | <b>31.57</b> | <b>921.36</b> | <b>2221.57</b> | <b>0.00</b> | <b>11861.45</b> |

# $\gamma$ -linolenic acid level increases to more than 11% in seed oil of *csd12a-3* x *csd15-1* double mutant

atty acid composition of the seed oil in seeds segregating homozygous *csd12a-3* x *csd15-1* double mutants



- $\gamma$ -linolenic acid (18:3n6) levels increase from about 3% (WT) to more than 11% in *csd12a-3* x *csd15-1* double mutant
- $\alpha$ -linolenic acid (18:3n3) levels drop from about 25% (WT) to less than 0.5% in *csd12a-3* x *csd15-1* double mutant
- $\gamma$ -linolenic acid levels in borage seed oil reach 24%. However, borage is difficult to grow as a crop and typical seed yields are much lower than those obtained for hemp (ca 0.4t/ha compared to >1.5t/ha)

# Generating new monoecious High Oleic Acid Hemp varieties

Source of *csfad2a-1* mutant allele:  
CNAP1HOH (Finola background)

Recurrent parents:

Earlina 08FC: monoecious seed oil  
variety with short growing cycle

USO-31: monoecious variety with  
early seed harvest and a crop with  
high fibre content; large seed



**MONOECIOUS**

Earlina 8 FC is the only variety exclusively bred for seed production in geographical situations in Northern Europe. Its very short growing cycle is perfectly adapted to late-spring and early-autumn weather conditions.

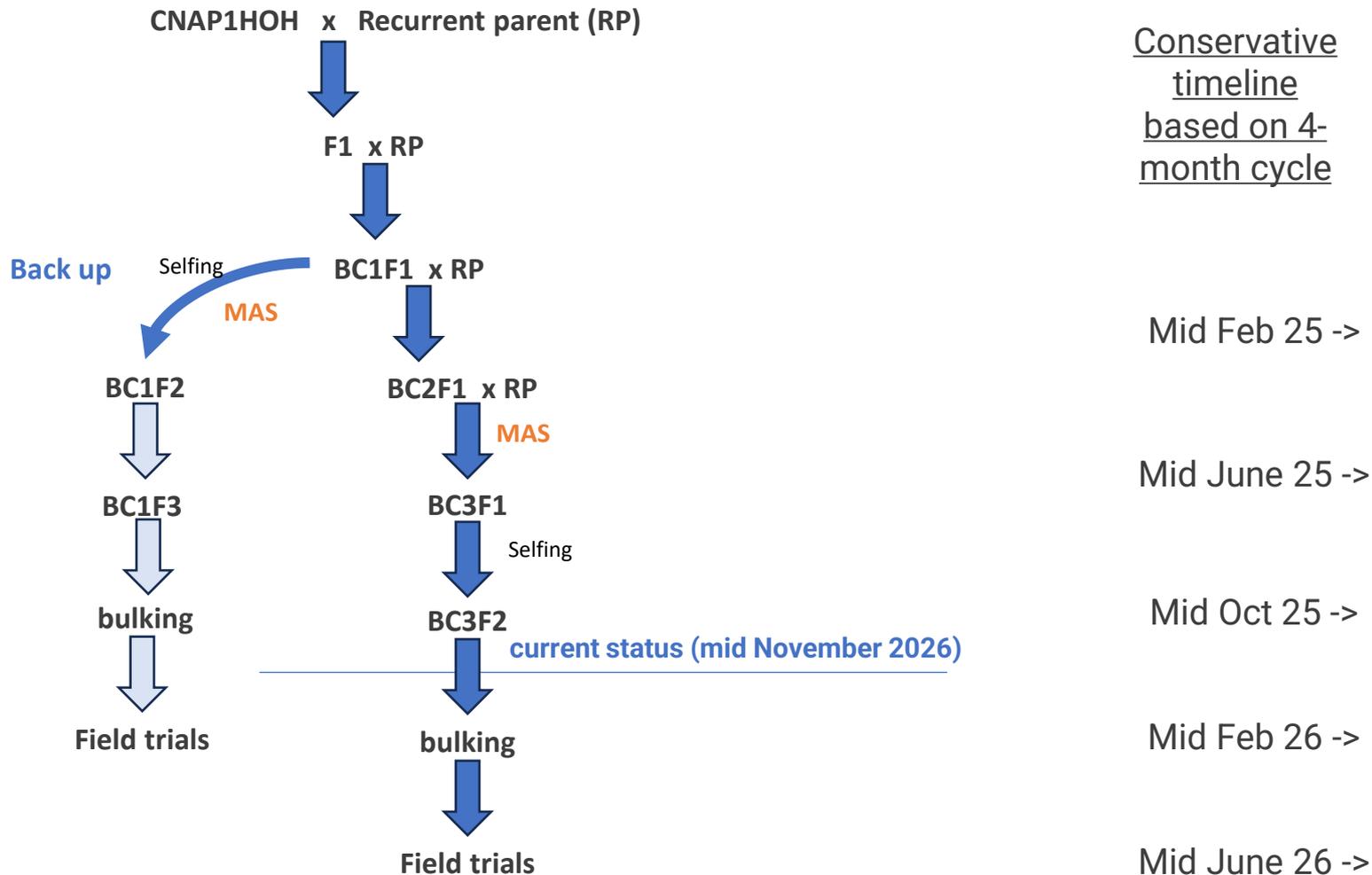


**MONOECIOUS**

**Growth cycle** : 122 to 127 days  
(for threshed crop harvesting, at the Le Mans latitude)

USO 31 is the perfect compromise between an early seed harvest and a crop with high fibre content. In Northern Europe, it is one of the only solutions to foresee a seed harvest in optimal weather conditions.

# Generating new monoecious High Oleic Acid Hemp varieties using Earlina 08FC as recurrent parent



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