Brassica carinata and Camelina sativa

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Ethiopian Mustard (*Brassica carinata*)

- Being developed as an industrial oil / bio-platform
- Biodiesel, biopesticide, plastics, polymers, pharmaceutical, and neutraceutical oils.
- Adapted to hotter, drier, longer growing season
Biodiesel feedstock, high protein meal

- High quality biodiesel, equal to or better than canola
- Low cloud point, oxidative stability
- Non-food oil, low ILUC feedstock

- High protein, low fibre meal
- 20% more crude protein than canola meal, approaching soybean levels
Why Biojet?

- Global GHG Reduction Goals for Aviation:
  - Carbon neutral growth from 2020
  - 50% emissions reduction by 2050

- Global Airline Agreements:
  - Industry-wide sign on to goals
  - IATA, CAAFI, ICAO driving efforts

- Proactive Efforts to Develop Global Value Chains
  - Airlines, OEMS, agencies all involved
• Higher yields of jet fuel
  • Higher probability of optimal cracking
  • Two jet fuel molecules (C8-C14) from one C22 acid
  • C18 oils can only produce one jet molecule
December 2012: Popular Science

Popular Science Magazine declared the 100% biofuel flight as

“one of the year's 25 most important scientific events”
Brassica carinata – Positive Agronomics

- Drought and heat tolerant
- Resistant to blackleg, tolerant to Alternaria
- Large seed size
- Shattering resistant
- Genetically diverse
Plants m\(^{-2}\) vs Brassica carinata yield

- **070760EM**
- **070768EM**
Seeds $m^{-2}$ vs Brassica carinata yield

070768EM

070760EM
**Brassica carinata** N response
(Adapted from Johnson et al. 2014. CJPS and unpublished data)

Assumptions:
- N fertilizer cost = 65 cents/lb
- Carinata Price = $ 9.50 / bushel
Brassica carinata – Agronomic challenges

- Weed control
- Late maturity
  - Maturity has been reduced by 5 to 7 days; however, lines are still 5 to 7 days later than B. napus.
  - Target area is southern Prairies, North and South Dakota, Montana.
- Susceptible to aster yellows
Brassica carinata Weed Control

• Major limitation
  – Grass weed control is not a problem – Quizalofop registered
  – Broadleaf weed control
    • Ethametsulfuron (Muster) registered – narrow broadleaf weed spectrum
    • Carinata has tolerance to dinotroaniline herbicides (soil applied). Trifluralin registered; Edge not registered.
    • Sulfentrazone (ppo inhibitor) – seeking minor use registration
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Herbicide Screening on *Brassica carinata* germplasm – Scott / Saskatoon 2009
Camelina sativa
### Seed quality and oil composition – a comparison

<table>
<thead>
<tr>
<th>Trait</th>
<th>Canola</th>
<th>Flax</th>
<th>Camelina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed oil content (%)</td>
<td>45</td>
<td>41.0</td>
<td>41.6</td>
</tr>
<tr>
<td>Protein content (%)</td>
<td>26.1</td>
<td>20.0</td>
<td>29.1</td>
</tr>
<tr>
<td>Fatty acid profile (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:1 (oleic)</td>
<td>60.3</td>
<td>20.0</td>
<td>14.0</td>
</tr>
<tr>
<td>C18:2 (linoleic)</td>
<td>18.0</td>
<td>16</td>
<td>18.0</td>
</tr>
<tr>
<td>C18:3 (linolenic)</td>
<td>11.6</td>
<td>53</td>
<td>36.0</td>
</tr>
<tr>
<td>C20:1 (gondoic)</td>
<td>&lt; 2</td>
<td>-</td>
<td>14.0</td>
</tr>
<tr>
<td>C22:1 (erucic)</td>
<td>-</td>
<td>-</td>
<td>2.5</td>
</tr>
</tbody>
</table>
Camelina sativa

• Desirable agronomic traits
  – Early maturity 80 to 100 days
  – Some drought, heat and frost tolerance
  – Resistant to flea beetles, blackleg, alternaria blackspot

Photo courtesy: Venkata Vakulabharanam
Saskatchewan Ministry of Agriculture
Camelina – Agronomic challenges

- Small seed – TKW ~ 1 gram (about 40% establishment rate)
- Broadleaf weed control
  - Tolerant to DNA’s; susceptible to pretty well every other broadleaf herbicide
- Susceptible to aster yellows, downy mildew, and sclerotinia
Outlook - Camelina as an industrial crop
10% of fossil oil is used in chemical industry

Products made from petrochemicals have the same value as the remaining 90% of fossil oil consumed as fuel

Plant oils can easily substitute for petrochemicals. Applications: soaps, paints, plastics, lubricants, hydraulic fluids, cosmetics, etc.

Added value can also be captured by plant oils!

Plant oils are renewable
The challenge: oil profile improvement for industrial applications

Fatty acid composition of camelina oil not optimal for oleochemical industry

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Camelina oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0 Palmitic acid</td>
<td>5.5%</td>
</tr>
<tr>
<td>18:0 Stearic acid</td>
<td>2.5%</td>
</tr>
<tr>
<td>18:1 Δ9 Oleic acid</td>
<td>14%</td>
</tr>
<tr>
<td>18:2 Δ9,12 Linoleic acid</td>
<td>18%</td>
</tr>
<tr>
<td>18:3 Δ9,12,15 Linolenic acid</td>
<td>36%</td>
</tr>
<tr>
<td>20:1 Δ11 Eicosenoic acid or Gondoic acid</td>
<td>14%</td>
</tr>
<tr>
<td>22:1 Δ13 Erucic acid</td>
<td>2.5%</td>
</tr>
<tr>
<td>12-OH 18:1 Δ9 Ricinoleic acid</td>
<td>Zero</td>
</tr>
</tbody>
</table>

- % PUFAs too high (C18:2, C18:3)
- % MUFAs too low (C18:1, C20:1, C22:1)
### Target fatty acid profiles of camelina seed for industrial uses

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Camelina oil</th>
<th>High Oleic</th>
<th>High Gondoic</th>
<th>High Ricinoleic</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0 Palmitic acid</td>
<td></td>
<td>5.5%</td>
<td>Low</td>
<td>8-10%</td>
</tr>
<tr>
<td>18:0 Stearic acid</td>
<td></td>
<td>2.5%</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>18:1 Δ9 Oleic acid</td>
<td>14%</td>
<td>70-80%</td>
<td>30-40%</td>
<td>55-60%</td>
</tr>
<tr>
<td>18:2 Δ9,12 Linoleic acid</td>
<td>18%</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>18:3 Δ9,12,15 Linolenic acid</td>
<td>36%</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>20:1 Δ11 Eicosenoic acid or Gondoic acid</td>
<td>14%</td>
<td>Zero</td>
<td>40-50%</td>
<td>Zero</td>
</tr>
<tr>
<td>22:1 Δ13 Erucic acid</td>
<td>2.5%</td>
<td>Zero</td>
<td>Low</td>
<td>Zero</td>
</tr>
<tr>
<td>12-OH 18:1 Δ9 Ricinoleic acid</td>
<td>Zero</td>
<td>Zero</td>
<td>Zero</td>
<td>25-30%</td>
</tr>
</tbody>
</table>

- Gondoic acid – jojoha oil
- Ricinoleic acid – castor oil
Metabolic Engineering *Camelina sativa* with Fish Oil-Like Levels of DHA

James R. Petrie, Pushkar Shrestha, Srinivas Belide, Yoko Kennedy, Geraldine Lester, Qing Liu, Uday K. Divi, Roger J. Mulder, Maged P. Mansour, Peter D. Nichols, Surinder P. Singh

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**Abstract**

*Background:* Omega-3 long-chain (≥C20) polyunsaturated fatty acids (n3 LC-PUFA) such as eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA) are critical for human health and development. Numerous studies have indicated that deficiencies in these fatty acids can increase the risk or severity of cardiovascular, inflammatory and other diseases or disorders. EPA and DHA are predominantly sourced from marine fish although the primary producers are microalgae. Much work has been done to engineer a sustainable land-based source of EPA and DHA to reduce pressure on fish stocks in meeting future demand, with previous studies describing the production of fish oil-like levels of DHA in the model plant species, *Arabidopsis thaliana*.

*Principal Findings:* In this study we describe the production of fish-oil-like levels (>12%) of DHA in the oilseed crop species *Camelina sativa* achieving a high ω3/ω6 ratio. The construct previously transformed in *Arabidopsis* as well as two modified construct versions designed to increase DHA production were used. DHA was found to be stable to at least the Tc generation and the EPA and DHA were found to be predominantly at the sn-1,3 positions of triacylglycerols. Transgenic and parental lines did not have different germination or seedling establishment rates.

*Conclusions:* DHA can be produced at fish-oil-like levels in industrially-relevant oilseed crop species using multi-gene construct designs which are stable over multiple generations. This study has implications for the future of sustainable EPA and DHA production from land-based sources.

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**UK Scientist Seeks GM Camelina Omega-3 Outdoor Trial**

Date Posted: January 27, 2014

*World Bulletin* -- The proposed trial - likely to generate controversy in a nation where GM foods have little public support - could start as early as May and will use Camelina plants engineered to produce seeds high in Omega-3 long chain fatty acids.

No GM crops are currently grown commercially in Britain and only two are licensed for cultivation in the European Union (EU).

No GM crops are currently grown commercially in Britain and only two - a pest-resistant type of maize and a potato with enhanced starch content - are licensed for cultivation in the European Union (EU).

But scientists at Britain’s agricultural lab Rothamsted Research have developed Camelina plants to produce Omega-3 fats that are known to be beneficial to health but normally found only in oils in increasingly limited fish stocks.

The idea, they told journalists at a briefing on their plans, is initially to supply the fish farming industry - which currently consumes around 80 percent of fish oils
Effect of Seeding Rate on Yield

Minimum seeding rate: 300 seeds/m²

Eric Johnson et al. unpublished data
Effect of Plant Emergence on Yield

Minimum plant population: **130 plants/m²** (42 % emergence)
Recommended seeding rate: **500 seeds/m² (5 lbs/acre)**
guarantees sufficient plant stands even at reduced emergence

Eric Johnson et al. unpublished data
N Response in Camelina

Assumptions:
N fertilizer cost = 45 cents/lb
Camelina Price = 18 cents/lb or $ 9.00 / bushel

Malhi, Johnson et al. 2014. Canadian Journal of Soil Science
N Response in Camelina – Indian Head 2009

0 kg/ha
75 kg/ha
150 kg/ha
200 kg/ha
Camelina Seeding Dates

- Why looking at different seeding dates?
  - Small seed
  - Not very competitive early on
- Early maturity
- Success with fall seeding in US and other places – will form rosette in fall if planted early enough
Camelina Seeding Date

- 5 fall and 3 spring seeding dates
- Scott, Swift Current, Indian Head
Scott 2010 Plant Density

Camelina Plant Density (LSD=59)

# plants/m^2

- 2-Oct
- 9-Oct
- 20-Oct
- 27-Oct
- 5-Nov
- 21-Apr
- 12-May
- 3-Jun

density

0 60 120 180 240
Scott 2010

Camelina Grain Yield (LSD=248)

Yield (kg/ha)

Scott

- 2-Oct
- 9-Oct
- 20-Oct
- 27-Oct
- 5-Nov
- 21-Apr
- 12-May
- 3-Jun
Fall
Test 88 - Biodiesel Potential of Alternative Species

• **Objective**
  – To evaluate the agronomic performance and the biodiesel quality of ten different oilseed species or cultivars.

• **Locations**
  – Lethbridge, Swift Current, Scott, Indian Head, Morden over 2 years
Crops / Cultivars Evaluated

1. *Brassica napus* canola cv. Invigor 5440
2. *Brassica rapa* canola cv ACS-C7
3. *Camelina sativa* cv. Calena
4. *Brassica carinata* ethiopian mustard (Common seed)
5. *Sinapis alba* yellow mustard cv. Andante
6. Canola quality *Brassica juncea* cv. Xceed 7784
7. *Brassica juncea* oriental mustard cv. Cutlass
8. Flax cv. Bethune
9. Soybean cv. LS0036RR
10. Soybean cv. OAC Prudence
Relative Yields (kg/ha) Camelina vs *Brassica napus*

<table>
<thead>
<tr>
<th>Location</th>
<th>Year</th>
<th><em>Brassica napus</em></th>
<th>Camelina</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lethbridge</td>
<td>2008</td>
<td>2850</td>
<td>4150</td>
<td>&gt;</td>
</tr>
<tr>
<td></td>
<td>2009</td>
<td>4040</td>
<td>2120</td>
<td>&lt;</td>
</tr>
<tr>
<td>Swift Current</td>
<td>2008</td>
<td>1780</td>
<td>1910</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td>2009</td>
<td>520</td>
<td>740</td>
<td>&gt;</td>
</tr>
<tr>
<td>Scott</td>
<td>2008</td>
<td>2720</td>
<td>1840</td>
<td>&lt;</td>
</tr>
<tr>
<td></td>
<td>2009</td>
<td>2260</td>
<td>2360</td>
<td>=</td>
</tr>
<tr>
<td>Indian Head</td>
<td>2008</td>
<td>3230</td>
<td>1530</td>
<td>&lt;</td>
</tr>
<tr>
<td>Morden*</td>
<td>2008</td>
<td>1730</td>
<td>1850</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td>2009</td>
<td>1890</td>
<td>1580</td>
<td>=</td>
</tr>
</tbody>
</table>

* Morden – highest yields obtained with soybean and flax
Weed Control in Camelina

• Options are limited
• Quizalifop registered for grass weed control; glyphosate registered for pre-harvest weed control
• Some growers using Trifluralin or Ethafluralin
Untreated  -  Imazmethabenz  -  Fluroxypyr-MCPA  -  2,4-D amine

MCPA amine  -  Bentazon  -  Bromoxynil  -  2,4-DB

Clopyralid  -  Glufosinate  -  Sulfentrazone  -  Imazamox/Imazethapyr
Progress in ALS (Group 2) resistance

Wild-type Untreated

Wild-type Treated with Refine SG 1X

Mutated Line Untreated

Mutated Line Treated with Refine SG 1X
Camelina – Future

- Well adapted to all soil zones on the prairies.
- Should be targeted to:
  - Gray
  - Dark Gray
  - Black
  - Dark Brown
  - Brown

Alberta
Saskatchewan
Manitoba

Early maturity
Drought, heat tolerance
Early Maturity - Facilitate Seeding of Winter Wheat
Summarize

• New crops
  – The next Cinderella crop? Who knows?
  – Timing of introduction to growers and market is critical
    • Credible agronomy will do nothing but help even it exposes “warts”
  – Weed control challenge for adoption of crops
    • High producer expectations for weed control
  – Strive for long-term sustained growth.
Acknowledgements

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