Utility of the *Arabidopsis* FAE1 and yeast SLC1-1 genes for improvements in erucic acid and oil content in rapeseed

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Abstract

High-erucic acid (HEA) *Brassica napus* cultivars are regaining interest in industrial contexts. Erucic acid and its derivatives are important renewable raw materials utilized in the manufacture of plastic films, in the synthesis of Nylon 13,13, and in the lubricant and emollient industries. Theoretically, the highest level of erucic acid that can be achieved by means of classical breeding is 66 mol%; however, using new approaches based on genetic engineering, it might be possible to develop a *B. napus* cultivar containing levels of erucic acid significantly above 66 mol% (> 80 mol%). In an attempt to increase the amounts of very-long-chain fatty acids (VLCFAs), and erucic acid in particular, in Canadian HEA *B. napus* cultivars, we have focused on two targets using a transgenic approach. We examined both the role/function of the *Arabidopsis thaliana* FAE1 (fatty acid elongase) gene by expressing it under the control of the seed-specific napin promoter in *B. napus* germplasm with analysis of the changes in VLCFA content in the seed oil of transgenic lines, and the performance of the yeast SLC1-1 (sphingolipid compensation mutant) in *B. napus* cv. Hero transgenic progeny in the field. Here, we report analyses of the contents of 22:1, total VLCFA and oil in the seed oil, as well as seed yield of the field-grown FAE1 and SLC1-1 *B. napus* cv. Hero progeny.

Introduction

High-erucic acid (HEA) *Brassica napus* cultivars are regaining interest for industrial purposes. Erucic acid (22:1) and its derivatives are important renewable raw materials used in plastic film manufacture, in the synthesis of Nylon 13,13, and in the lubricant and emollient industries [1]. Theoretically, the highest level of erucic acid that can be achieved through classical breeding is 66 mol%. This limitation has been attributed to the specificity of the *B. napus* sn-2-acyltransferase, which excludes erucic acid from the sn-2 position of triacylglycerols [2].

However, using new approaches based on genetic engineering, it may be possible to develop a *B. napus* cultivar containing erucic acid levels significantly above 66 mol% (more than 80 mol%). A *B. napus* line containing high proportions of erucic acid would significantly reduce processing costs and could meet the forecast demand for HEA oil as a renewable, environmentally friendly industrial feedstock [3,4].

Continuing our efforts to increase the amounts of very-long-chain fatty acids (VLCFAs), and 22:1 in particular, in Canadian HEA *B. napus* cultivars, we focused on two targets...
using a transgenic approach. To address the supply of erucyl moieties for oil synthesis, we examined the role and function of the *Arabidopsis thaliana* gene FAE1 (fatty acid elongase) by expressing it under the control of the seed-specific napin promoter in *B. napus* HEA germplasm and analysed the changes in VLCFA content in the seed oil of transgenic lines. The best FAE1 transgenic lines were field tested. We also tested the performance of the yeast SLC1-1 (sphingolipid compensation mutant) in *B. napus* cv. Hero [5] transgenic progeny in the field. Here we report detailed analyses of 22:1 content, total VLCFA content and oil content in the seed oil as well as seed yield of the field-grown FAE1 and SLC1-1 *B. napus* cv. Hero progeny.

**Results**

**Expression of Arabidopsis FAE1 gene in HEA B. napus cv. Hero: increasing the pool of VLCFAs**

The analyses of fatty acid composition in seed oil of T₂ Hero FAE1 transgenic lines and wild type controls are shown in Figure 1. Proportions of 22:1 content, total VLCFA content and oil content in the seed oil as well as seed yield of the field-grown FAE1 and SLC1-1 *B. napus* cv. Hero progeny.

**Figure 1**

The accumulation of 22:1 and VLCFAs in T₂ mature seeds of cv. Hero non-transformed wild-type controls (WT) and Hero FAE1 transgenic lines

Fatty acid proportions are shown as the % (w/w) of total fatty acids. Each bar represents the mean ± S.D. of ten samples, with single seeds being analysed in each sample.

**Table 1**

Proportions of erucic acid, total VLCFAs and oil content in seed of non-transformed wild-type control plants (H-ntCon) and T₃ seed of selected FAE1 transgenic lines of *B. napus* cv. Hero from field-trial studies

Results in square brackets for transgenic lines are the % increase relative to the non-transformed wild-type Hero control. DW, dry weight.

<table>
<thead>
<tr>
<th>Line</th>
<th>% 22:1 (w/w) [% increase]</th>
<th>% Total VLCFAs (w/w) [% increase]</th>
<th>Oil content (% of DW) [% increase]</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-ntCon</td>
<td>48.1</td>
<td>57.6</td>
<td>44.2</td>
</tr>
<tr>
<td>H-10-2</td>
<td>59.1 [22.9]</td>
<td>67.4 [17.0]</td>
<td>47.2 [6.8]</td>
</tr>
<tr>
<td>H-10-5</td>
<td>56.5 [17.5]</td>
<td>64.7 [12.3]</td>
<td>46.2 [4.5]</td>
</tr>
<tr>
<td>H-10-6</td>
<td>56.7 [17.9]</td>
<td>64.8 [12.5]</td>
<td>46.5 [5.2]</td>
</tr>
<tr>
<td>H-10-10</td>
<td>57.1 [18.7]</td>
<td>65.2 [13.1]</td>
<td>44.7 [1.1]</td>
</tr>
<tr>
<td>H-14-7</td>
<td>56.2 [16.8]</td>
<td>63.9 [10.9]</td>
<td>49.0 [10.9]</td>
</tr>
</tbody>
</table>
22:1 and VLCFAs are shown as % (w/w) of total fatty acids. In our best Hero T2 transgenic lines, proportions of 22:1 were in the range of 48–53% while wild-type control lines had an average of 43% erucic acid.

The capacity for VLCFA biosynthesis (elongase activity) was assayed in vitro using homogenates from developing seeds at 30–35 days after pollination from Hero FAE1 transgenic lines and Hero wild-type controls. The FAE1 transgenic progeny showed 22–100% increases in total elongase activity (results not shown).

Field trials with FAE1 B. napus cv. Hero transgenic progeny
Mature T2 seed was collected from field-grown lines and analysed for 22:1 and oil content. The best Hero FAE1 transgenic lines showed 8–11% increases in 22:1 proportions and 2–4.8% increases in oil content, in comparison with the wild-type controls (Table 1).

Field trials with SLC1-1 B. napus cv. Hero transgenic progeny
The T2 progeny in the best six field-grown lines showed 2.8–5.6% increases, representing net increases of 6.7–13.5% overall in oil content on a dry-weight basis. There were also 2.4–7% (w/w) increases in 22:1 proportions, representing net increases of 5.2–15% overall. Yield (g/plot) was consistently higher in these transgenic lines (Table 2).

Conclusions
Thus far we have expressed separately two main target genes: (i) a gene encoding an sn-2-acyltransferase from yeast (SLC1-1) and (ii) a gene encoding a seed-specific condensing enzyme from A. thaliana (FAE1) in the HEA B. napus cv. Hero. Individually expressed, both genes contributed to increases in VLCFAs in seed oil. However, neither gene alone could boost the amounts of erucic acid to the target level of 80%. We have demonstrated that, in rapeseed, the proportion of 22:1 is limited by both 22:1 synthesis and its subsequent incorporation into triacylglycerols.

Accordingly, we are in the process of returning to breeding and selection, initiating crosses between our best SLC1-1 and FAE1 cv. Hero transgenic lines. By combining both traits we will eventually produce lines that are superior to parental transgenic lines with respect to both 22:1 content and oil content.

References

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