The overexpression of a mutant *Brassica* 3-hydroxy-3-methylglutaryl-CoA synthase A in transgenic model plant, tobacco, promotes growth and seed yield

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http://dx.plos.org/10.1371/journal.pone.0098264
Importance of increasing seed yield in crops

● Seeds ➔ Food

major food source for man and domesticated animals
rice, wheat and maize comprise ~60% food
(http://www.fao.org/docrep/u8480e/u8480e07.htm)

● World population ~7 billion (2014)


~13.5% of global population go hungry
(http://www.who.int/bulletin/volumes/89/2/11-020211/en/)

● Identification of genes that control seed yield is useful
The overexpression of a mutant *Brassica* 3-hydroxy-3-methylglutaryl-CoA synthase A (HMGS), enzyme in isoprenoid pathway in transgenic model plant, *tobacco*, promotes growth and seed yield.
**Isoprenoids**

- Large, diverse, natural products
- **Phytosterols**: lower cholesterol
- Gibberellic acid, abscisic acid (ABA) & cytokinins: **growth & development**
- Carotenoids and chlorophyll: **photosynthesis**
Isoprenoid biosynthesis in plants

Hypothesis:
Overexpression of HMGS will increase phytosterols (end-products)

Enzymes are shown in bold. Pathway inside the mitochondria and plastid are boxed. Arrows between cytosolic and plastid compartments represent metabolic flow between them (greater arrow for more flux). Abbreviations: ABA, abscisic acid; AACT, acetacetyl-CoA thiolase; BR6OX2, brassinosteroid-6-oxidase 2; CYP710A1, sterol C-22 desaturase; CYP85A1, cytochrome P450 monooxygenase; DMAPP, dimethylallyl diphosphate; DWF1, delta-24 sterol reductase; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; DXS, 1-deoxy-D-xylulose 5-phosphate synthase; FPP, farnesyl diphosphate; GA-3-P, glyceraldehyde-3-phosphate; FPPS, farnesyl diphosphate synthase; GAs, gibberellins; GGPP, geranylgeranyl diphosphate; GGPPS, geranylgeranyl diphosphate synthase; GPP, geranyl diphosphate; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; IPP, isopentenyl diphosphate; IPP1, isopentenyl/dimethylallyl diphosphate isomerase; Q10, coenzyme Q10; SMT, sterol methyltransferase; SQS, squalene synthase.
Background on BjHMGS

Cloned *BjHMGS* cDNA encoding 3-hydroxy-3-methylglutaryl-CoA synthase (*BjHMGS*)
from an edible plant, *Brassica juncea* (Brassicaceae family)

Characterization of BjHMGS

- BjHMGS is developmentally regulated and stress-responsive
  
  Nagegowda et al. (2005) *Planta* 221: 844-856

- Expression of recombinant wild-type & mutant HMGS in bacteria
  
  mutant S359A displayed a 10-fold higher enzyme activity
  

- Crystal structure of BjHMGS
  
  Pojer et al. (2006) *Proc Natl Acad Sci USA* 103: 11491-11496
Will the overexpression of BjHMGS1 in plants enhance phytosterol production?

Tested the hypothesis in Arabidopsis, model plant with short generation time
Brassicaceae family closely-related to Brassica

Results:
Overexpression of wild-type & mutant BjHMGS1 in Arabidopsis
up-regulated genes in sterol biosynthesis & enhanced sterol production and stress tolerance
Will the **positive effects** be conserved in a phylogenetically less-related species?

Tested in **tobacco (model plant)** from distant Solanaceae family

**Tobacco is easy to transform & regenerate**

**Plant of choice in plant genetic engineering experiments**

easy to obtain transgenic plants;

reduces time to obtain & analyse progeny from derived transgenic lines
**Sterol increase** was conserved in transgenic tobacco HMGS-OE seedlings (S) and leaves (L).

Campesterol, stigmasterol, \(\beta\)-sitosterol and total sterol content in 20-d-old seedlings and leaves from 2-m-old plants.

Values are means ± SD (\(n = 5\)); H, higher than vector (pSa13)-transformants; 
* \(P < 0.05\); ** \(P < 0.01\) by Student’s t-test.

DW, dry weight; Bars represent SD.

Overexpression of BjHMGS1 in transgenic tobacco

(phylogenetically-distant species)

*also resulted in*

increased plant growth, pod size & seed yield

<table>
<thead>
<tr>
<th>210-d-old flowering plants</th>
<th>vector</th>
<th>OE-WT</th>
<th>OE-S359A</th>
</tr>
</thead>
<tbody>
<tr>
<td>210-d-old flowering plants</td>
<td>pSa13</td>
<td>401</td>
<td>402</td>
</tr>
<tr>
<td></td>
<td>10 cm</td>
<td>210-d</td>
<td>76 cm</td>
</tr>
</tbody>
</table>

14-d-old tobacco HMGS-OE seedlings grow better than vector(pSa13)-transformants

A Seedlings 14-d post-germination
Bar = 1 cm
pSa13, the vector-transformed control
OE-wtBjHMGS1 ("401" and "402")
OE-S359A ("603" and "606")

B Root length measurements of 14-d-old seedlings
Values are mean ± SD (n=30); H, higher than control

C Dry weight determination of 14-d-old seedlings
Values are mean ± SD (n=30); H, higher than control

a, significant difference between HMGS-OE and vector transformant
b, significant difference between OE-wtBjHMGS1 and OE-359A
80-d-old HMGS-OEs grow better than vector(pSa13)-transformants

Representative tobacco plants photographed 80 days after germination
Bar = 10cm

pSa13, vector control
OE-WT, overexpressing wild-type HMGS
OE-S359A, overexpressing mutant HMGS S359A

Statistical analyses on height of plants at different growth stage
Values are mean ± SD (n=30)
Bars are SD; H, higher than control
a, significant difference between HMGS-OE and vector transformant
b, significant difference between OE-wtBjHMGS1 and OE-359A
**, *P* < 0.01 by Student’s t-test
98-d-old-tobacco HMGS-OEs show better growth

**pSa13**, the vector-transformed control  
**OE-wtBjHMGS1** (lines “401”, “402” and “404”)  
**OE-S359A** (lines “602”, “603” and “606”)

Bar (A and C) represents 10 cm  
Values (B and D) are mean ± SD (n=6); Bars are SD; **, \( P < 0.01 \); *, \( P < 0.05 \); ** and *, significantly higher than control, by the Student’s \( t \)-test

Tobacco HMGS-OEs showed increase in pod size and seed yield

A Phenotype of tobacco pods. Scale bar = 1 cm.

B Average dry weight per pod.

C Average seed number per pod.

Thirty independent readings were taken for each line. Values are means ± SD, n = 30.; **, P < 0.01; *, P < 0.05 by the Student’s t-test.

pSa13, the vector-transformed control

OE-wtBjHMGS1 (lines 401” and “402”)

OE-S359A (lines “603” and “606”)

Liao et al. (2014) PLOS ONE 9(5): e98264
RT-PCR on expression of genes encoding “downstream” enzymes

**Enzymes are shown in bold.** Pathway inside the mitochondria and plastid are boxed. Arrows between cytosolic and plastid compartments represent metabolic flow between them (greater arrow for more flux). Abbreviations: ABA, abscisic acid; AACT, acetoacetyl-CoA thiolase; BR6OX2, brassinosteroid-6-oxidase 2; CYP710A1, sterol C-22 desaturase; CYP85A1, cytochrome P450 monoxygenase; DMAPP, dimethylallyl diphosphate; DWF1, delta-24 sterol reductase; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; DXS, 1-deoxy-D-xylulose 5-phosphate synthase; FPP, farnesyl diphosphate; GA-3-P, glyceraldehyde-3-phosphate; IPP, isopentenyl diphosphate; Q10, coenzyme Q10; SMT, sterol methyltransferase; SQS, squalene synthase.
Effects of HMGS on isoprenoid biosynthesis gene expression in tobacco HMGS-OEs

Expression of **HMGS downstream** genes by qRT-PCR

Total RNA was extracted from 20-d-old tobacco seedlings. H, value higher than the control ($P<0.05$, Student’s $t$-test); L, value lower than the control ($P<0.05$, Student’s $t$-test). Values are means ± SD (n=3).

**pSa13**, vector-transformed control

**OE-wtBjHMGS1** (lines “401”, “402” and “404”)

**OE-S359A** (lines “602”, “603” and “606”)

Effects of HMGS on the expression of plastidial GGPPSs in tobacco HMGS-OEs

qRT-PCR in 20-d-old tobacco HMGS-OE seedlings

Liao et al. (2014) PLOS ONE 9(5): e98264
RT-PCR on expression of genes encoding “downstream” enzymes

Tobacco overexpressing wild-type BjHMGS1 and S359A showed induction of native NtHMGR1, NtIPI2, NtSQS, NtSMT1-2, NtSMT2-1, NtSMT2-2 and NtCYP85A1 resulting in enhanced sterol content, improved growth and enhanced seed yield (26.5% for OE-wtBjHMGS1; 67% for OE-S359A)

NtSQS greatly upreg in S359A

Summary


Isoprenoid pathway can be engineered using HMGS S359A to enhance phytosterol content, growth and seed production in phylogenetically-distant tobacco

<table>
<thead>
<tr>
<th>Parameters</th>
<th>% increase in OE-wtBjHMGS1 over vector control</th>
<th>% increase in OE-S359A over vector control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytosterol content (20-d-old seedlings)</td>
<td>4.4</td>
<td>25.7</td>
</tr>
<tr>
<td>Phytosterol content (leaves from 60-d-old plants)</td>
<td>12.1</td>
<td>18.7</td>
</tr>
<tr>
<td>Height (98-d-old plants)</td>
<td>90.9</td>
<td>97.3</td>
</tr>
<tr>
<td>Height (210-d-old plants)</td>
<td>21.0</td>
<td>45.2</td>
</tr>
<tr>
<td>Seed yield</td>
<td>26.5</td>
<td>67.4</td>
</tr>
<tr>
<td>Pod dry weight</td>
<td>22.6</td>
<td>50.2</td>
</tr>
</tbody>
</table>