Plant Biotechnology Journal

Plant Biotechnology Journal (2013) **11**, pp. 355–361 doi: 10.1111/pbi.12021

Suppression of the *SUGAR-DEPENDENT1* triacylglycerol lipase family during seed development enhances oil yield in oilseed rape (*Brassica napus* L.)

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Received 14 August 2012;
revised 27 September 2012;
accepted 3 October 2012.
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Keywords: triacylglycerol lipase, seed maturation, oil content, oilseed rape.

Summary

Increasing the productivity of oilseed crops is an important challenge for plant breeders and biotechnologists. To date, attempts to increase oil production in seeds via metabolic pathway engineering have focused on boosting synthetic capacity. However, in the tissues of many organisms, it is well established that oil levels are determined by both anabolism and catabolism. Indeed, the oil content of rapeseed (*Brassica napus* L.) has been reported to decline by approximately 10% in the final stage of development, as the seeds desiccate. Here, we show that RNAi suppression of the *SUGAR-DEPENDENT1* triacylglycerol lipase gene family during seed development results in up to an 8% gain in oil yield on either a seed, plant or unit area basis in the greenhouse, with very little adverse impact on seed vigour. Suppression of lipolysis could therefore constitute a new method for enhancing oil yield in oilseed crops.

Introduction

Vegetable oils (triacylglycerols) are an important global commodity. They form a significant part of the nutritional intake of humans and livestock, serve as feedstock for the chemical industry and also provide a renewable source of energy in the form of biofuels (Durrett *et al.*, 2008; Dyer *et al.*, 2008). World production has increased by more than 50% in the past decade and now is in excess of 145 million metric tons per year (http://www.fas.usda.gov/oilseeds/). Improving the yield of oil crops is therefore widely acknowledged to be an important agronomic goal (Lu *et al.*, 2011), and conventional breeding, combined with better crop management, continues to make incremental improvements (Weselake *et al.*, 2009). Alternatively, one transgenic approach to achieve this objective is to boost the metabolic flux of carbon to oil within the developing seed (Weselake *et al.*, 2009). A number of studies have established that seed oil content can be increased by the overexpression of individual oil biosynthetic enzymes (Jako *et al.*, 2001; Roesler *et al.*, 1997; Vigeolas *et al.*, 2007; Zou *et al.*, 1997), or transcriptional 'master' regulators that govern the expression of multiple enzymes (Cernac and Benning, 2004; Shen *et al.*, 2010).

Interestingly, in many eukaryotic tissues, the content of oil is governed by the dynamic balance between both synthesis and breakdown and a deficiency in triacylglycerol hydrolysis has been shown to cause an increase in oil deposition (Grönke *et al.*, 2005; Kurat *et al.*, 2006; Zimmermann *et al.*, 2004). The role of oil catabolism in determining the content of oil-rich plant tissues such as those of seeds has received little attention, although there is a literature to suggest that catabolic pathways are active during seed development (Graham, 2008). In *Arabidopsis thaliana*, *Brassica napus*, *Crambe abyssinica* and *Nicotiana tabacum*, oil content peaks late in the maturation phase, but data in several studies suggest that it then drops as the seed desiccates on the plant (Baud *et al.*, 2002; Gurr *et al.*, 1972; Molina *et al.*, 2008; Murphy and Cummins, 1989; Tomlinson *et al.*, 2004). Chia *et al.* (2005) investigated this phenomenon in *B. napus* (oilseed rape) and showed that the amount of oil in seeds falls by more than 10% over the course of desiccation. Oilseed rape is the third most important oil crop, after palm (*Elaeis guineensis*) and soybean (*Glycine max*), and supplies more than 23 million metric tons of oil, with an estimated commodity value in excess of $30 billion (http://www.fas.usda.gov/oilseeds). Even a gain in oil yield of approximately 10% would add substantial value to the crop. Furthermore, seed desiccation occurs after the supply of nutrients from the mother plant has effectively ceased (Chia *et al.*, 2005). Because maternal resources are already committed to the seed, prevention of oil loss during desiccation may be considered very likely to enhance the oil yield in the field.

We previously identified a small family of triacylglycerol lipase genes in Arabidopsis, consisting of *SUGAR-DEPENDENT1* (*SDP1*) and *SDP1-LIKE* (*SDP1L*), which are responsible for initiating oil breakdown in the seeds following germination (Eastmond, 2006; Kelly *et al.*, 2011). SDP1 and SDP1L are members of an unorthodox group of lipases that are related to patatin from potato (*Solanum tuberosum*), but contain a divergent active site (Scherer *et al.*, 2010). Well-characterized examples include *Homo sapiens* adipose triglyceride lipase (Zimmermann *et al.*, 2004), *Drosophila melanogaster* Brummer (Grönke *et al.*, 2005) and *Saccharomyces cerevisiae* triacylglycerol lipase 3, 4 and 5 (Athenstaedt and Daum, 2005; Kurat *et al.*, 2006). *SDP1* appears to be expressed in all Arabidopsis tissues, but most strongly during seed maturation, prompting us to question whether genes from this family might be responsible for oil turnover in developing seeds, as well as during post-germinative growth (Eastmond, 2006). Here, we investigated this question directly in oilseed rape using a transgenic approach.

Results

Rapeseed oil content declines during seed desiccation

To investigate whether *SDP1* triacylglycerol lipase family genes play a role in oil turnover during oilseed rape seed development, we chose to use a commercial open-pollinated spring variety called Kumily (Lantmännen SW Seed AB) as an experimental system. To characterize the pattern of oil accumulation over the course of seed development in this variety, plants were grown under both greenhouse and field conditions. Seeds were harvested from siliques on the primary raceme and their developmental stage was scored morphologically (Figure 1a) using a scale similar to that described previously by Chia *et al.* (2005). This scale includes seeds at early, early–mid and mid–late phases in oil accumulation, at the onset of desiccation and at maturity. The total fatty acid content of Kumily seeds (as determined by gas chromatography) increased up until the onset of desiccation, when the content reached approximately 2.3 mg of total fatty acids/seed. It then declined significantly by maturity [$P < 0.05$, least significant difference (LSD) test], equating to a net loss of approximately 10% in the greenhouse and approximately 9% in the field (Figure 1b).

RNAi strategy suppresses SDP1 family during seed desiccation

In order to suppress *SDP1* triacylglycerol lipase family function in Kumily seeds, three cDNAs were identified in oilseed rape (GenBank accession numbers GN078290, GN078281 and GN078283), which are >80% identical to Arabidopsis *SDP1* (At5g04040) and *SDP1L* (At3g57140) at the nucleotide level. A very highly conserved 300-bp region of the 3′-open reading frame of GN078283 was then chosen to create an *SDP1* family RNA interference (RNAi) construct (Wesley *et al.*, 2001) driven by the USP promoter from *Vicia faba*, which is active throughout embryo maturation (Bäumlein *et al.*, 1991) (Figure S1). The construct was transformed into Kumily via *Agrobacterium*-mediated transformation (Moloney *et al.*, 1989), and approximately 30 low copy number primary transformants were selected by quantitative PCR (qPCR; Bubner and Baldwin, 2004). Based on preliminary analysis of seed from the primary transformants (Zank *et al.*, 2008), five lines (24AS, 31AS, 92AS, 62AS and 72AS) were taken to the third generation (T_3) and homozygous plants were identified by segregation analysis. *SDP1* family transcript levels were measured in desiccating embryos of these plants (Figure 1a) using qPCR, and they were repressed by between 10- and 30-fold relative to either wild type (WT) or a transformed empty vector control (EVC; Figure 2).

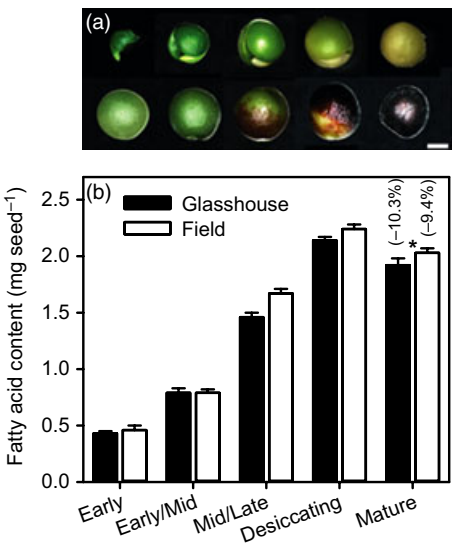

Figure 1 The total fatty acid content of *Brassica napus* cv. Kumily seeds declines during seed desiccation. (a) Representative images of seed and embryo morphology at five developmental stages selected for lipid analysis (Chia *et al.*, 2005). Scale bar = 1 mm. (b) Total fatty acid content of seeds from field- and greenhouse-grown plants at each developmental stage. Plants were grown between March and August. The values are expressed in mg/seed and are the mean ± SE ($n = 12$). Asterisk denotes a statistically significant [$P < 0.05$, least significant difference (LSD) test] difference between desiccating and mature ($F_{4,88} = 614.03$, SED = 0.043 on 88 df, LSD at 5% = 0.085 for the main effect of developmental stage). There was also a main effect of environment ($F_{1,22} = 9.43$, SED = 0.028 on 22 df, $P = 0.006$) with means 1.44 (field) and 1.35 (greenhouse), but no interaction between developmental stage and environment ($P = 0.121$, *F*-test). The values in brackets are the mean percentage decrease between desiccating and mature.

SDP1 RNAi enhances seed oil content

To determine whether the oil content of the seeds was affected by SDP1 RNAi, 12 homozygous T_3 plants of each genotype were grown in the greenhouse in a randomized block design. Seeds harvested from each plant were then dried and weighed, and their oil and protein contents were assayed using near-infrared reflectance spectroscopy (Hom *et al.*, 2007; Table 1). The seed oil content (as a % of seed weight) was significantly increased in all five SDP1 RNAi lines when compared to WT or EVC ($P < 0.001$, LSD test), and the gain ranged between 3% and 8%. In contrast, seed protein content was significantly reduced ($P < 0.05$, LSD test) in all five, but by a comparatively small amount (<4%). The total weight of seeds produced by the plants was taken as a measure of seed yield and did not differ significantly between genotypes ($P = 0.928$, *F*-test). However, the oil yield, calculated by multiplying the total weight of seeds from each plant by the % oil content, was significantly different ($P < 0.05$, LSD test) in three of the SDP1 RNAi lines. In these lines (62AS, 72AS and 92AS),

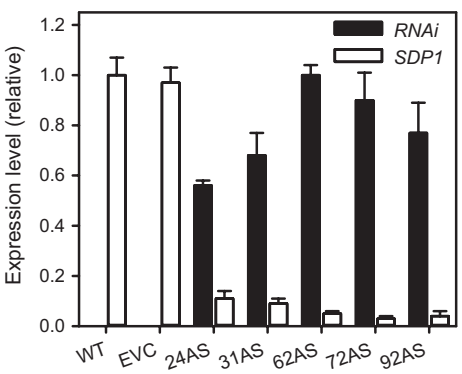

Figure 2 RNAi suppresses *SDP1* transcript abundance during Kumily seed maturation. The values are the mean ± SE of measurements taken using quantitative RT-PCR on desiccating seeds (Figure 1a) from four plants of each genotype grown in the greenhouse and are expressed as arbitrary units. RNAi primers were used to detect the *SDP1* RNAi transcript, and SDP1 primer was used to detect the *SDP1* transcripts. *ACTIN2* expression was used as a control for normalization. WT, wild type; EVC, empty vector control; AS, independent homozygous T_3 *SDP1* RNAi line; ND, not detected.

Table 1 RNAi-mediated suppression of *SDP1* during Kumily seed maturation increases seed oil content per plant

Genotype	Oil content (%)	Protein content (%)	Seed yield (g/plant)	Oil yield (g/plant)	Protein yield (g/plant)
WT	42.36 ± 0.12	21.78 ± 0.10	16.21 ± 0.32	6.87 ± 0.13	3.53 ± 0.06
EVC	42.20 ± 0.09	21.87 ± 0.10	15.96 ± 0.17	6.74 ± 0.07	3.49 ± 0.04
24AS	43.84 ± 0.10[c]	21.39 ± 0.10[a]	16.31 ± 0.36	7.15 ± 0.16	3.49 ± 0.08
31AS	44.58 ± 0.15[c]	21.39 ± 0.08[a]	16.08 ± 0.35	7.17 ± 0.16	3.44 ± 0.07
62AS	44.92 ± 0.15[c]	21.40 ± 0.14[a]	16.40 ± 0.28	7.36 ± 0.13[a]	3.51 ± 0.06
72AS	45.86 ± 0.13[c]	21.04 ± 0.10[c]	16.36 ± 0.39	7.51 ± 0.19[b]	3.44 ± 0.09
92AS	45.21 ± 0.18[c]	21.01 ± 0.13[c]	16.46 ± 0.15	7.44 ± 0.07[b]	3.46 ± 0.04
$F_{6,66}$	106.42	8.42	0.31	3.97	0.24
SED_{66}	0.193	0.161	0.454	0.205	0.098
LSD (5%)	0.386	0.321	0.907	0.410	0.196
LSD (1%)	0.513	0.426	1.204	0.545	0.260
LSD (0.1%)	0.666	0.553	1.564	0.708	0.338

Values are the mean ± SE of measurements on seeds from individual plants (n = 12) of each genotype grown in the greenhouse.

The superscript letters denote a statistically significant difference from WT (LSD test), where significant differences between genotypes were detected (F-test).

[a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$.

WT, wild type; EVC, empty vector control; AS, independent homozygous T_3 SDP1 RNAi line; F, F-statistic on df in subscript; SED, SE of the difference between means on df in subscript; LSD, least significant difference.

which also had the highest % oil content and the lowest *SDP1* transcript levels (Figure 2), there was a gain in oil yield of between 7% and 9%.

To determine whether an oil yield increase could be reproduced on a per unit area basis, small plots of WT and 72AS plants were grown in the greenhouse at a density of 120 plants m^2, which simulates field conditions (Table 2). The yield of seeds per m^2 plot was not significantly different between WT and 72AS (P = 0.636, F-test). However, the oil yield per m^2 was significantly greater (P = 0.022, LSD test), increasing by approximately 9%. The seed yield per unit area that was obtained in the greenhouse (approximately 270 g m^2) was within the range obtained for the same variety grown in UK field trials in 2010 (http://www.hgca.com). To investigate when, during seed development, SDP1 RNAi exerts an effect on seed oil content, a time-course experiment was performed (Figure 3). A significant difference in seed total fatty acid content was only detected between WT and SDP1 RNAi lines at maturity ($P < 0.05$, LSD test), with 72AS and 92AS having 7%–8% greater fatty acid content at this stage. These data suggest that SDP1 RNAi mainly impacts oil turnover during seed desiccation, although a small effect in the earlier phase of maturation cannot be discounted (Figure 3). The WT (Kumily) is a 'canola' or '00' variety with characteristically high oleic acid and very low erucic acid contents in its seed oil. The analysis of fatty acid composition using gas chromatography confirmed that SDP1 RNAi had no effect on this trait in mature seeds (Table S1), and no obvious differences in fatty acid composition were detected throughout seed maturation.

SDP1 RNAi has very little impact on seed vigour

Using SDP1 RNAi as a strategy to boost oil yield is beneficial so long as seed vigour is not compromised, because slow and uneven seedling establishment is known to adversely affect crop yield (Finch-Savage *et al.*, 2010). SDP1 is necessary to support normal seedling establishment in Arabidopsis, although growth inhibition is only apparent when oil breakdown is quite substantially restricted (Eastmond, 2006; Kelly *et al.*, 2011). The RNAi strategy was designed to suppress *SDP1* family gene function during seed maturation, but not following germination. The USP promoter is highly active throughout embryo maturation (Bäumlein *et al.*, 1991). However, like many other 'seed-specific' promoters, a low level of activity has been observed in some other tissues (Bäumlein *et al.*, 1991; Zakharov *et al.*, 2004). The analysis of total fatty acid breakdown following seed germination suggested a very slight initial delay in SDP1 RNAi lines versus WT ($P < 0.05$, LSD test), but no significant difference in peak rate

Table 2 RNAi-mediated suppression of *SDP1* during Kumily seed maturation increases oil yield per unit area

Genotype	Oil content (%)	Protein content (%)	Seed yield (g m^2)	Oil yield (g m^2)	Protein yield (g m^2)
WT	42.16 ± 0.10	21.39 ± 0.05	265.7 ± 5.7	112.0 ± 2.39	56.84 ± 1.27
72AS	45.39 ± 0.10[c]	20.70 ± 0.08[c]	270.1 ± 6.7	122.6 ± 2.85[a]	55.90 ± 1.28
$F_{1,9}$	409.31	35.36	0.24	7.60	0.26
SED_9	0.159	0.116	9.02	3.84	1.838

Values are the mean ± SE of measurements on seeds from 1-m^2 plots (n = 6) of each genotype grown in the greenhouse.

The superscript letters denote a statistically significant difference from WT (LSD test), where significant differences between genotypes were detected (F-test).

[a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$.

WT, wild type; AS, independent homozygous T_3 SDP1 RNAi line; F, F-statistic on df in subscript; SED, SE of the difference between means on df in subscript.

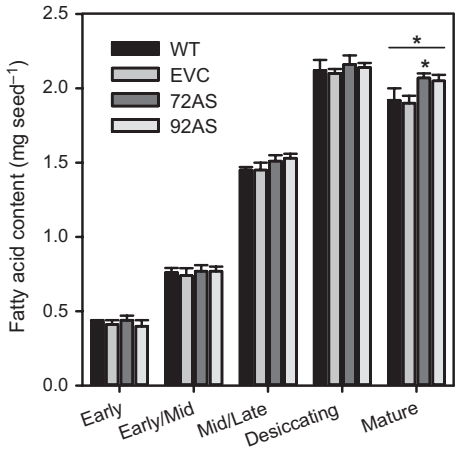

Figure 3 Suppression of *SDP1* during Kumily seed maturation reduces the decline in fatty acid content over the course of desiccation. The values are total fatty acid content in mg/seed and are the mean ± SE of measurements taken on seeds from individual plants of each genotype grown in the greenhouse (n = 12). Seeds at each of the five developmental stages were selected based on their morphology (Figure 1a). Asterisk above the line denotes a statistically significant [$P < 0.05$, least significant difference (LSD) test] overall difference between desiccating and mature ($F_{4,176}$ = 1152.7, SED = 0.031 on 176 df, LSD at 5% = 0.062 for the effect of developmental stage). Asterisk below the line denotes a statistically significant difference from wild type (WT) at mature stage ($P < 0.05$, LSD test).

between genotypes (P = 0.892, F-test; Table 3). Studies on seed vigour in small-seeded crop species have consistently identified germination rate and initial root and shoot growth rate to be good predictors of performance in the field (Finch-Savage *et al.*, 2010). No significant difference ($P < 0.05$, F-tests) was observed between WT and SDP1 RNAi lines for any of these three traits when they were assayed under laboratory conditions (Table 3). Finally, homozygous T_3 SDP1 RNAi seed batches were stored at room temperature and humidity for up to 4 years and only those that were more than 2 years old exhibited a significant reduction in germination rate versus WT ($P < 0.05$, LSD test), and this difference remained comparatively small (Table 4).

Discussion

Here, we have shown that suppressing oil turnover during rapeseed maturation using an SDP1 RNAi strategy can result in up to a approximately 8% increase in oil yield on a per seed, per plant or a per unit planted area basis, in the greenhouse, with relatively little adverse impact on seed vigour. Multisite field trials would ultimately be necessary to fully validate the commercial potential of this approach, including assessing the impact of certain agricultural practices such as swathing and desiccant application. However, our data suggest that this technology is likely to deliver small, but consistent, oil yield improvements in the field. SDP1 RNAi primarily blocks oil loss during seed desiccation. This loss occurs in greenhouse and field conditions and takes place after the nutrient supply from the mother plant has effectively ceased (Baud *et al.*, 2002; Chia *et al.*, 2005).

The increase in percentage oil content observed in SDP1 RNAi seeds is accompanied by a decrease in percentage protein content, although this decrease is not quantitatively proportional (Table 1). The protein content of rapeseed is important because the protein meal, generated as a by-product of oil extraction, is sold as feed for livestock. However, oil is by far the most valuable constituent of the seed and has a commodity value that is more than 4.5 times greater than the meal (http://www.fas.usda.gov/oilseeds/). In many countries, farmers receive a bonus at market, based on the percentage oil content of their crop. For example, if the results we present in Table 2 translated to the field, then a farmer in the UK would gain an additional approximately 4.5% of the contract price in oil bonus, with no deduction for the marginal difference in the protein content of the seed meal (http://www.hgca.com). This equates to a gain of approximately £17/ton of seed at current market prices.

It is important to consider that SDP1 RNAi might also be compatible with other transgenic approaches that are designed to increase oil biosynthetic capacity in the seed, such as overexpression of oil biosynthetic enzymes (Jako *et al.*, 2001; Roesler *et al.*, 1997; Zou *et al.*, 1997) or their transcriptional activators (Cernac and Benning, 2004; Shen *et al.*, 2010). In this case, an additive effect on oil yield might be achieved. Several such approaches to increase biosynthetic capacity have already

Table 3 Suppression of *SDP1* during Kumily seed maturation does not impair the rate of fatty acid breakdown (FAB) following germination or seed vigour

Genotype	Rate of FAB (μg/day)	FAB at 24 h (%)	Germination (h)	Shoot growth (mm/h)	Root growth (mm/h)
WT	0.44 ± 0.05	5.4 ± 1.6	22.6 ± 0.3	0.207 ± 0.02	0.260 ± 0.03
EVC	0.40 ± 0.04	5.1 ± 0.8	23.1 ± 0.5	0.210 ± 0.03	0.264 ± 0.02
72AS	0.39 ± 0.03	1.0 ± 1.1*	22.3 ± 0.3	0.207 ± 0.02	0.264 ± 0.03
92AS	0.41 ± 0.03	1.2 ± 0.6*	22.7 ± 0.3	0.210 ± 0.02	0.265 ± 0.02
$F_{3,15}$	0.20	4.19	0.53	0.01	0.005
SED_{15}	0.0619	1.666	0.600	0.0339	0.0441
LSD (5%)	0.1319	3.550	1.279	0.0722	0.0939

Seed vigour assays were performed as described by Finch-Savage *et al.* (2010), and the values are the mean ± SE on measurements taken on ten seed from six plants of each genotype (n = 6). Measurements of total fatty acid contents were also taken on pools of ten seeds from six plants of each genotype (n = 6), and the percentage of fatty acid breakdown (FAB) after 24 h and the peak rate of FAB between 48 and 72 h were determined.

WT, wild type; EVC, empty vector control; AS, independent homozygous T_3 *SDP1 RNAi* line; LSD, least significant difference.

*Statistically significant difference from WT ($P < 0.05$, LSD test), where significant differences between genotypes were detected (F-test).

Table 4 Suppression of *SDP1* during Kumily seed maturation only affects the germination rate after prolonged storage

	Time to 50% germination (h)			
Genotype	1 year old	2 years old	3 years old	4 years old
WT	22.6 ± 0.4	20.9 ± 0.3	21.7 ± 0.5	24.1 ± 0.3
EVC	23.0 ± 0.3	21.6 ± 0.7	22.0 ± 0.3	23.9 ± 0.3
72AS	22.3 ± 0.2	21.9 ± 0.3	25.8 ± 0.8*	31.7 ± 0.4*
92AS	22.7 ± 0.4	21.3 ± 0.4	24.7 ± 0.5*	35.2 ± 0.3*

Germination assays were performed on seed batches harvested between 1 and 4 years ago using the method described by Finch-Savage *et al.* (2010), and the values are the mean ± SE on measurements taken on 60 seed from six plants of each genotype ($n = 6$).

WT, wild type; EVC, empty vector control; AS, independent homozygous T_3 *SDP1 RNAi* line.

*Statistically significant ($P < 0.05$, LSD test) difference from WT ($F_{9,75} = 32.16$, SED = 0.677 on 75 df, LSD at 5% = 1.349).

been reported to increase rapeseed oil content by between 5% and 20% per seed, depending on the mechanism and study (Weselake *et al.*, 2009). Although there have only been a few cases where it has been demonstrated that this translates into an oil yield increase per unit area, as would be required in the field (Weselake *et al.*, 2009).

The precise role of oil turnover during rapeseed desiccation is not fully understood. It may simply result from some overlap in the developmental programmes that govern late seed maturation and post-germinative seedling development. Alternatively, Chia *et al.* (2005) proposed that it provides a carbon source to support metabolic activity during desiccation, promoting continued synthesis of seed storage proteins and late embryogenesis–abundant proteins. Radiolabel feeding experiments do indicate that the oil is used to fuel respiration, and to a lesser extent amino acid synthesis, but not gluconeogenesis (Chia *et al.*, 2005). Although our analysis of SDP1 RNAi lines suggests that the physiological consequences of impairing oil breakdown in developing seeds are only slight, we did observe a small reduction in seed longevity.

Given that SDP1 RNAi seeds appear to gain oil content partly at the expense of protein, it is possible that a metabolic readjustment occurs in the desiccating seeds, whereby protein and amino acids provide a carbon source to compensate for the relative inaccessibility of oil. There is already evidence that this occurs during early post-germinative growth in Arabidopsis mutants that are deficient in the glyoxylate cycle (Cornah *et al.*, 2004). Finally, there is some evidence to suggest that oil turnover may actually be a widespread occurrence in desiccating seeds from various species (Chia *et al.*, 2005), and therefore, the suppression of *SDP1* family genes might have a broader application in other oilseed crops.

Experimental procedures

Plant growth conditions and experimental design

For the analysis of seed from greenhouse-grown plants, wild type and transgenic *Brassica napus* (L.) cv. Kumily were planted either individually in 3-L pots or in plots of approximately 120 in 1 × 1 × 0.3 m trays containing Scotts Levington M2 compost and grown to seed. Two neighbouring 20-m^2 greenhouse compartments with air conditioning (18 °C day/12 °C night) and 16 h of supplemental illumination (approximately 300 μmol/m^2/s at canopy height) were used. Individual plants were arranged into a 12-block, one-way randomized design with one plant of each genotype per block and randomized within each block. Six blocks were placed in each compartment. Plots of each genotype were arranged alternately with six in each compartment. The position of blocks and plots was rotated within each compartment every week. For the analysis of seed from field-grown plants, the material was harvested from within a 1-m^2 area. For all the experiments performed on developing seeds, the material was harvested from siliques situated around the middle of the primary raceme. Seed vigour assays were performed as described by Finch-Savage *et al.* (2010). Seeds were applied to vertically orientated wet filter paper and placed in the dark at 15 °C, and time to germination, 15 mm shoot length and 40 mm root length were scored visually. There were ten seeds per replicate and six replicates per genotype. The initial rate of shoot and root growth was calculated by subtracting the time to germination. Seeds/seedlings were also harvested at 24-h periods to measure the total fatty acid content and determine the peak rate of fatty acid breakdown.

Construct preparation and transformation

In order to suppress storage oil hydrolysis in developing Kumily embryos, an RNAi construct was generated (Zank *et al.*, 2008). Three cDNAs were identified in *B. napus* (GenBank accession numbers GN078290, GN078281 and GN078283) that are >80% identical to Arabidopsis SDP1 (At5g04040) and SDP1L (At3g57140) at the nucleotide level, and a highly conserved 300-bp region of the 3′-open reading frame of GN078283 was selected for RNAi (Figure S1a). This region was fused in direct and reverse-complement orientation to the ends of a linker sequence from *Physcomitrella patens*. The RNAi construct was then fused with the USP promoter from *Vicia faba*, which drives seed-specific expression (Bäumlein *et al.*, 1991), and with the OCS terminator from *Agrobacterium tumefaciens*. The expression construct was cloned into the vector pENTR-A and used in a Gateway® reaction with an empty pENTR-B and pENTR-C and a pSUN2-based destination vector (Cheng *et al.*, 2010) to create the binary plant transformation vector (Figure S1b) *B. napus* cv. Kumily was transformed using the method described by Moloney *et al.* (1989) except that imidazolinone resistance was used for positive selection in tissue culture. Low copy number lines were identified by performing quantitative PCR on genomic DNA (Bubner and Baldwin, 2004) using primers to the RNAi construct, as described below for transcript measurements. An empty vector control (EVC) line with a single T-DNA insertion (confirmed by Southern blot) was used as a reference.

Transcript measurement

DNase-treated total RNA was isolated from *B. napus* embryos using the RNeasy kit from Qiagen with the modifications described by Eastmond (2006). The synthesis of single-stranded cDNA was performed using SuperScript II RNase H-reverse transcriptase from Invitrogen. Quantitative PCR was performed with an ICycler (Bio-Rad) using qPCR Mastermix Plus (Eurogentec), according to the manufacturer's instructions, and the data were analysed with ICycler IQ5 software. The primer pairs were SDP1 (5′-GTCCTCTTCTGCAAATCAATGCT-3′ and 5′-GAGGAA CCAGTGGAGGAGGAA-3′), RNAi (5′-CCATCTCCCTCTCAGGTA-TATCTATCTG-3′ and 5′-CATAGGCGTCTCGCATATCTCA-3′) and

ACTIN2 (5′-ACGAGCTACCTGACGGACAAG-3′ and 5′-GAGCGACGGCTGGAAGAGTA-3′).

Metabolite analysis

A Buchi NIRFlex N-500 Solids Near-Infrared Reflectance Spectrometer (NIRS) with vial add-on (BUCHI UK Ltd, Oldham, UK) was used for seed oil and protein determination in conjunction with the INGOT Rapeseed calibration for vial format (Aunir Ltd, Towcester, UK). For each individual plant, three 15 × 45 mm glass vials of seeds were measured and the mean values used for subsequent calculations. In the case of plots, twenty glass vials of seeds were measured and the mean values used for subsequent calculations. The accuracy of the NIRS method for determining *B. napus* seed oil content was supported by a strong correlation ($r > 0.95$) with data obtained using an MQC23 bench top NMR machine (Oxford Instruments, Oxford, UK). For the determination of fatty acid content and composition, gas chromatographic analysis of fatty acid methyl esters was carried out using the method described by Barker *et al.* (2007) with the following modifications. For each sample, ten or twenty seeds or seedlings were frozen in liquid nitrogen and freeze-dried, before being homogenized and incubated together with 5 mg of tripentadecanoin standard in 5 mL of 1 N methanolic HCl at 85 °C for 12 h.

Statistical analysis

The number of replicates (n) and the standard error (SE) of the mean are shown for all measurements. One-way analysis of variance (ANOVA) was used to assess the differences between genotypes for the measurements of seed percentage oil and percentage protein content, weight, fatty acid breakdown, shoot and root growth and germination rate. A split-plot in time (two-way) ANOVA was used for seed fatty acid content during development to take account of the repeated measures from plants over the time course (early, early–mid, mid–late, mature and desiccating), having checked for a consistent correlation structure over consecutive time points using a linear mixed model. Two-way ANOVA was used for seed fatty acid content comparing different environments over development, and for germination rate of seed from genotypes harvested in different years. All ANOVAs took account of the randomized block design structure of the different experiments and inspection of residuals concluded that no transformation of data was required. Following significant ($P < 0.05$) F-test results for the main effects of, or interactions between, genotype and developmental stage (or harvest year) factors, means of interest were compared using the appropriate LSD value at the 5% ($P = 0.05$) level of significance, on the corresponding degrees of freedom (df). The GenStat (2011, 14th edition; ©VSN International Ltd, Hemel Hempstead, UK) statistical system was used for these analyses.

Acknowledgements

We wish to thank BASF Plant Science GmbH (Limburgerhof, Germany) for cloning *SDP1* homologues in *B. napus*, performing transformation and providing the T_1 transgenic material used in this study. We are also grateful to the Horticultural Services staff at the University of Warwick for plant husbandry and Dr Christian Craddock and Dr Nicolette Adams for their assistance in harvesting and threshing seeds. This work was supported by the UK Biotechnology and Biological Sciences Research Council through grant BB/E022197/1. PJE is the named inventor for patent US8093452.

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Supporting information

Additional Supporting information may be found in the online version of this article:

Figure S1 Details of the *SDP1 RNAi* construct used for suppression of oil breakdown in Kumily.

Table S1 Suppression of *SDP1* during Kumily seed maturation does not substantially alter fatty acid composition.