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ARTICLE

Enhanced Seed Carotenoid Levels and Branching in Transgenic *Brassica napus* Expressing the *Arabidopsis miR156b* Gene

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The *Arabidopsis AtmiR156b* gene was expressed in *Brassica napus* under the control of the cauliflower mosaic virus (CaMV) 35S promoter and the seed-specific napin promoter. Seed carotenoid levels, branching habit, seed yield, and seed weight were examined in the transgenic *B. napus*. Our results demonstrated that constitutive expression of *AtmiR156b* in *B. napus* resulted in enhanced levels of seed lutein and β-carotene and a 2-fold increase in the number of flowering shoots, whereas *AtmiR156b* driven by the napin promoter did not affect these traits. This suggested that enhancement of seed quality and shoot branching are both related to *AtmiR156b* expression patterns. Seed yield and seed weight varied significantly within the transgenic lines. However, one line was found to have enhanced seed carotenoid levels but unchanged seed weight or yield. These data suggest that *AtmiR156b* gene expression could be applied in plant breeding initiatives for enhancing carotenoid production in canola and other crop species.

KEYWORDS: ***Arabidopsis AtmiR156b***; ***Brassica napus*** **transformation; seed carotenoids; branching habit**

1. INTRODUCTION

The *Arabidopsis* microRNA *miR156* regulates a network of genes by targeting 10 *SPL* (*SQUAMOSA PROMOTER BINDING PROTEIN LIKE*) transcriptional factors (*1*−*3*), some of which regulate leaf primordium initiation and transition from the vegetative to the reproductive stage (*4*−*7*). *MiR156* overexpression in *Arabidopsis* increases the number of rosette leaves and shoot branches (*1*). The recent discovery of strigolactones, plant branching inhibitors derived from carotenoids (*8*, *9*), revealed that plant branching is related to carotenoid metabolism. Together, these reports suggest a link among *miR156*, carotenoid metabolism, and shoot branching with the potential to affect seed yield and quality in crops.

Brassica napus (canola) is a commercially valuable seed crop, which is grown throughout the world for animal feed, vegetable oil, and biodiesel. Substantial effort has been directed toward the genetic enhancement of the nutritional composition of *B. napus* seeds (*10*). Carotenoids are valuable components in *B. napus* seeds and have potential as supplements in livestock feed. Plant carotenoids are also important in the human diet. The consumption of β-carotene, a precursor of vitamin A, is closely related to human health owing to its role as an antioxidant (*11*−*14*).

Carotenoid biosynthesis in plants has been well studied and has been reviewed in seed and nongreen tissues (*15*). Significant enhancement of carotenoid accumulation has been achieved in several different plants by manipulating genes directly involved in the carotenoid biosynthetic pathway or more distantly related genes (*16*−*18*). More recently, suppression of the negative regulator of light-mediated responses in tomato, *DE-ETIOLATED-1* (*DET1*), resulted in a simultaneous increase in the level of carotenoids and flavonoids (*19*). RNAi-mediated suppression of *DET1* in *B. napus* increased seed carotenoid accumulation and reduced antinutritional sinapine esters while maintaining normal plant morphology and seed productivity (*20*). These studies indicate the potential of using specific regulatory genes to enhance carotenoid production and improve other plant traits.

In this study, the *Arabidopsis miR156b* was expressed in *B. napus* using the constitutive CaMV 35S promoter and the seed-specific napin promoter (*21*), and the effect on seed carotenoids, branching, and seed production evaluated.

2. MATERIALS AND METHODS

2.1. Plant Material and Growing Conditions. The *B. napus* doubled haploid line, DH12075, was used as recipient germplasm for transformation with the *AtmiR156b* transgene. All transgenic lines were grown in plastic pots (diameter 12.5 cm) containing Co−Co Mix potting growth media as prepared before (*20*) in a controlled environment greenhouse (16 h light/8 h dark, 20 °C/17 °C). About 50 days after seeding, the large basal leaves were removed for better plant management. Upon flowering, plants were bagged to prevent accidental cross-pollination, and mature seeds were subsequently harvested.

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Table 1. Primers Used in the Experimental Procedures

primer	sequence	note
miR156b_XbaI_F	5′-*tctaga*GTAAGACACGTGTAGAAATCTTC-3′	*Xba*I site italicized. For gene cloning.
miR156b_R	5′-ACTTCAGGGTGAAGCACATTAG-3′	For gene cloning
35S_F3	5′- CAATCCCACTATCCTTCGCAAGACCC-3′	PCR screening
Napin_F3	5′-gc*agatct*AAGCTTGCTTTCTTCATCGGTGATTGATTCC-3′	italicized *Bgl*II site, for PCR screening
GUS_2R	5′-CAG ACG TTG CCC GCA TAA TTA C-3′	PCR screening
qmiR156_R	5′-CTCTTTCTGTCAGTTGCCTATCT-3′	PCR screening and transcript quantification
qmiR156b_F	5′-CAGAAGAGAGTGAGCACATGCAG-3′	transcript quantification

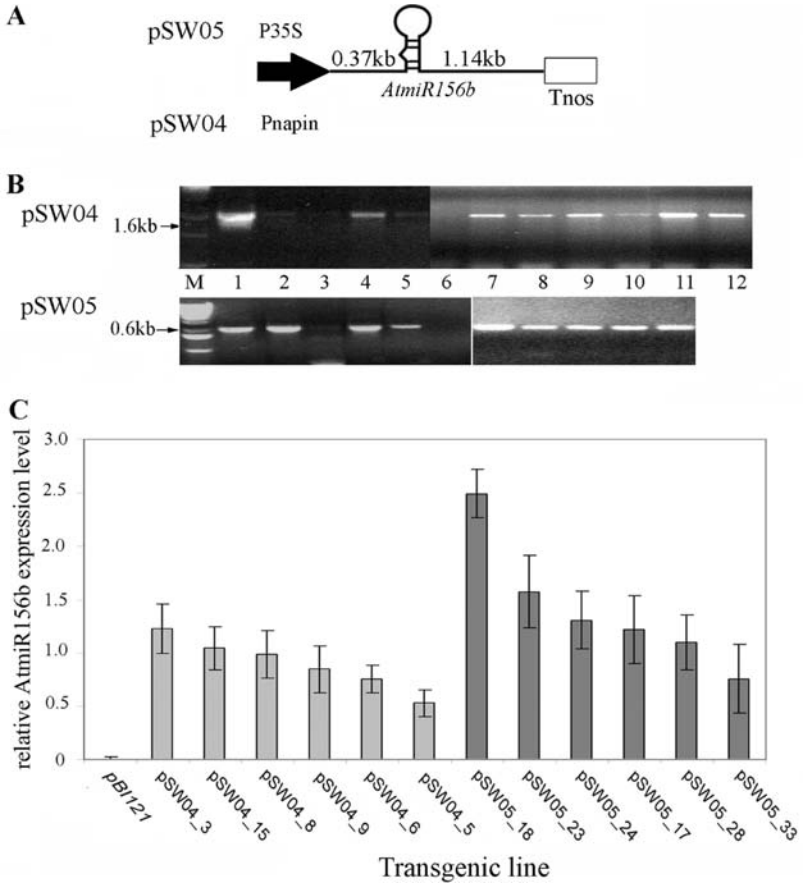

Figure 1. Constitutive and seed specific expression of pSW04 and pSW05 vectors (**A**) for generating transgenic *B. napus*. PCR-detected transgene presence (**B**) and transgene expression levels relative to that of reference gene 18S rRNA (**C**) in transgenic plants are also shown. P35S, cauliflower mosaic virus 35S promoter; *AtmiR156*, *Arabidopsis* miR156b gene; Tnos, nopaline synthase terminator; Pnapin, *B. napus* napin promoter. For pSW04: M, DNA molecular weight marker; lane 1, plasmid DNA carrying the transgene *AtmiR156*; lane 6, wild type plant; lanes 2−5 and 7−12, independent transgenic plant lines (lanes 2, 3, and 5 with very faint bands). For pSW05: M, DNA molecular weight marker; lane 1, plasmid DNA carrying the transgene; lane 6, wild type plant; lanes 2−5 and 7−11, independent transgenic plant lines (lane 3 does not show the typical transgene pattern; therefore, it may not be transgenic). Error bars of transgene expression levels represent the standard deviation among six replicates.

2.2. Plasmid Construction and Plant Transformation. The *AtmiR156b* gene was isolated by PCR amplification according to Schwab et al. (*1*) from 20 day old plants of *A. thaliana* ecotype Columbia using specific primers designed to include additional *Xba*I and *Sac*I sites at the 5′ and 3′ ends, respectively (**Table 1**). The gene was cloned into the pCR2.1-TOPO TA cloning vector (Invitrogen Inc., Mississauga, Canada) for sequencing and then excised and inserted into the *Xba*I/*Sac*I sites in pBI121 (Clontech, Mountain View, CA) downstream from the CaMV 35S promoter. Here, the *gusA* gene was replaced by *AtmiR156b*, and the resultant plant expression construct was designated pSW05 (**Figure 1A**). In construct pSW04, the 35S promoter in pSW05, was replaced by a *B. napus* napin promoter (*21*). Additionally, the pBI121 vector carrying the *gusA* gene was used to generate transgenic control plants. *Agrobacterium tumefaciens* (GV3101pMP90) harboring these constructs was used to transform *B. napus* DH12075 cotyledon explants as described before (*22*). Timentin (450 mg/L) was used to blot the *Agrobacterium* infected plant materials immediately after infection and also added to the selection media to eliminate *Agrobacterium* throughout the selection process. Regenerated plantlets that survived kanamycin (25 mg/L) selection were transplanted into a greenhouse. PCR amplification with one promoter-specific primer and one gene-specific primer (**Table 1**), followed by sequencing of the PCR product, was conducted to ensure there were no selection escapes. These in addition to the analysis of the transgene mRNA eliminated any false PCR positives arising from *Agrobacteruium* contamination. For pBI121 transformants, staining of leaves for β-glucuronidase (GUS) activity was also performed (*23*) to confirm transgene presence. For each construct, approximately 20 independent T_0 transgenic plants were analyzed.

2.3. DNA and RNA Extraction. Total plant genomic DNA was extracted from leaves using DNeasy Plant Mini Kit (Qiagen, Mississauga Canada). Total RNA was extracted from the mature seeds of all transgenic plants according to Shi and Bressan (*24*).

2.4. Transgene Transcript Quantification. *AtmiR156* transcript levels in transgenic plants were quantified using reverse transcription−quantitative polymerase chain reaction (RT-qPCR) and gene-specific primers (**Table 1**) designed to amplify the precursor transcript of *AtmiR156b*. First-strand cDNA synthesis and real time qPCR analysis using SYBR Green qPCR Master Mix (Cat No. 11735-040, Invitrogen, Carlsbad, CA) and Quantum RNA Universal 18S Internal Standard (Applied Biosystems/Ambion, Austin, TX) as the internal reference gene were performed as previously described (*20*). Control PCR reactions without cDNA templates were also performed for each primer pair. Because pBI121transgenic plants used as the control did not contain *AtmiR156b* and all PCR reactions displayed efficiencies around 100%, the relative expression level of the transgene to that of 18S rRNA for each independent transgenic line was calculated using $2^{-\Delta Ct}$, in which ΔCt means the difference in cycle number at the threshold between the target transgene and the reference gene. All samples were assayed in triplicate from two independent RNA samples and the mean expression values of all the replicates determined.

2.5. Plant Morphology Traits. On the basis of transgene transcript levels and seed carotenoid quantification in T_0 transgenic plants, five to six T_1 plants per independent transgenic event were used to evaluate branching and flowering phenotypes for the pSW05 and pSW04 constructs. Germinated T_1 plants were rescreened by PCR to confirm the presence of the transgene when the fourth to fifth true leaf was produced. The number of primary and flowering branches, seed yield, and seed weight were recorded for each plant. The means for all five T_1 plants per independent transformation event were analyzed by ANOVA and a Tukey statistical test using Origin 8.1 software (OriginLab Co., Northampton, MA, USA).

2.6. Carotenoid Extraction and Quantification. Seed carotenoid extraction and analysis on T_0 seeds using high performance liquid chromatography (HPLC) were carried out according to a protocol used in this laboratory (*25*). Compounds were identified by their retention time and absorption spectra compared to those of known standards. For each independent transgenic event, individual (lutein, β-carotene, violaxanthin, and β-crytoxanthin) and total carotenoids were determined for triplicate seed samples and means analyzed using ANOVA and a Tukey statistical test.

3. RESULTS

3.1. Generation of Transgenic *B. napus* Plants. CaMV 35S or napin promoter cassettes driving the *AtmiR156b* (**Figure 1A**) and a control pBI121 cassette driving the *gusA* gene were introduced into *B. napus* DH12075 by *Agrobacterium*-mediated gene transformation. Approximately 20 in vitro plantlets surviving kanamycin selection (per construct) were transplanted into soil in the greenhouse. PCR amplification using primers specific to each promoter and to the *AtmiR156b* gene showed products with expected sizes in pSW05 plants (35S) and pSW04 plants (napin) (**Figure 1B**) and was confirmed by sequencing. Plants carrying the pBI121 construct were verified by staining leaves for GUS activity (data not shown). *AtmiR156b* was expressed in the leaves of lines transformed with pSW05 and seeds of lines transformed with pSW04. Expression relative to the reference gene 18S rRNA ranged from 0.5−1.2-fold for pSW04 and around 1.5-fold for pSW05 (**Figure 1C**). Such expression levels of *AtmiR156b* in dry seeds in transgenic *B. napus* were relatively substantial considering the high abundance of 18S rRNA molecules (1000−1000000-fold calculated on the basis of the ΔCt) compared to the mRNA level of a normal housekeeping gene as detected by Nicot et al. (*26*). The *AtmiR156b* transcript in control pBI121 plants was undetectable.

3.2. Effect of *AtmiR156b* on Morphology and Seed Yield in Transgenic *B. napus*. The majority of pSW05 plants constitutively expressing *AtmiR156b* exhibited dramatic morphological changes, while pSW04 plants with seed-specific expression of the gene were phenotypically indistinct from control plants (**Figure 2**). In pSW05 transformants, most axillary buds along the main stems sprouted and developed into axillary branches

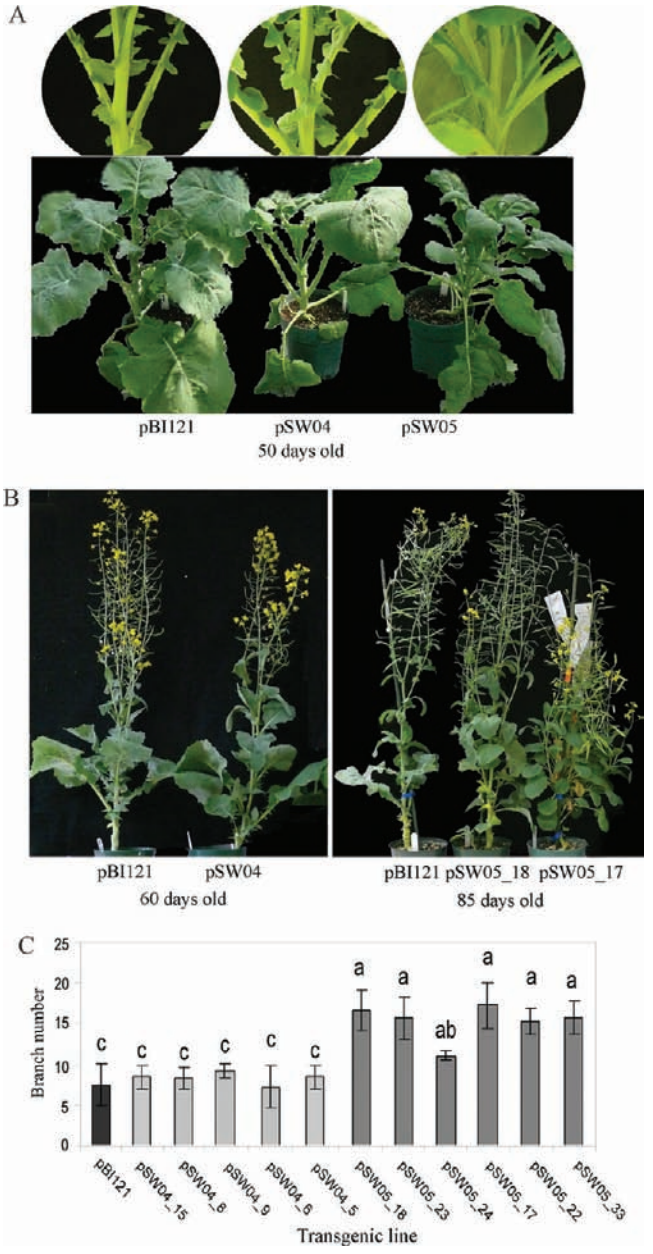

Figure 2. Enhanced branching phenotype associated with transgenic *B. napus* expressing *AtmiR156b* under the control of the 35S promoter at both vegetative (**A**) and reproductive stages (**B** and **C**). Bars with different letters represent statistically different means, $p \leq 0.05$. Error lines represent the standard deviation of five replicate measurements. pSW05_17 and pSW05_18 in **B** show the range of growth phenotypes observed.

over the period of vegetative growth (**Figure 2A**), which made the plants appear bushy. Variation in plant height was also found among pSW05 transformants. Approximately 10% of the pSW05 plants became very short and bushy. An additional 10% of pSW05 plants grew as tall as control plants, but with more branches. No significant differences in branching were found between the pBI121 control and the T_1 pSW04 lines (8.2 ± 2.7 per plant, mean value for all tested lines) (**Figure 2C**).

The onset of flowering in most pSW05 transgenic lines was similar to that of pBI121 control plants. However, enhanced reproductive branching was observed for the pSW05 plants which resulted in a much longer flowering period (> 2 months longer than control plants). T_1 85 day-old pSW05 transformants showed

a 2-fold greater number of flowering branches (15.3 ± 5.1 per plant, mean value for all tested lines) than the pBI121 control plants (7.5 ± 4.7 per plant, mean value for all tested plants) ($p < 0.05$) (**Figure 2B** and **C**). No significant difference in seed yield occurred between plants harboring pSW04 (6.15 ± 1.61 g/plant), pSW05 (4.02 ± 1.64 g/plant), or pBI121 (5.83 ± 1. 09 g/plant) ($p < 0.05$), although large variations in seed yield per plant were observed in some transgenic lines and in one case, pSW05-33, plants showed a slight reduction in seed yield (**Figure 3A**). Seeds expressing *AtmiR156b* exhibited a significantly lower weight per 100 seeds than control seeds, except for plants SW04-9 and pSW05-33 ($p < 0.05$), which had a seed weight similar to that of the control line (**Figure 3B**).

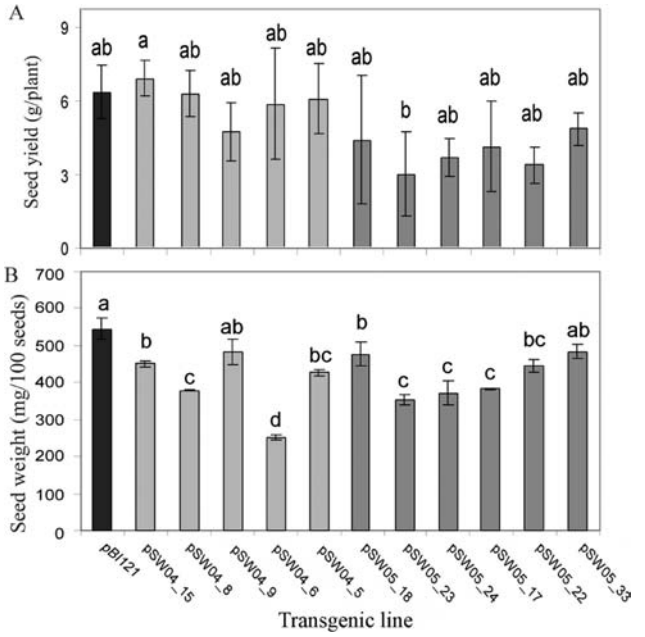

Figure 3. Seed yield (**A**) and seed weight (**B**) in *B. napus* lines expressing *AtmiR156b*. Bars with different letters represent statistically different means ($p \leq 0.05$). Error bars represent standard deviation of the means of 4 replicates. pSW04 lines, *napin* promoter driven *AtmiR156b*; pSW05 lines, CaMV *35S* promoter driven *AtmiR156b*.

3.3. Carotenoid Levels in Transgenic Seeds. Levels of the two major seed carotenoids, lutein and β-carotene, were quantified in mature T_0 seeds of *AtmiR156b* and pBI121 transgenic plants. Violaxanthin, β-cryptoxanthin, and other minor carotenoid compounds were not detected in mature seeds. Compared to control plants, the sum of the two detectable compounds in the seeds carrying pSW05 was increased 1.3−2-fold (**Figure 4**). The major carotenoid, lutein, increased in a manner similar to that of total carotenoids. However, a substantial change was found for the minor carotenoid, β-carotene, which increased up to 4.5-fold (**Figure 4**). Compared to pBI121-carrying seeds, the levels of lutein, β-carotene, and total carotenoids in mature seeds carrying pSW04 remained unchanged (**Figure 4**). This suggests that pSW05 was more effective than pSW04 at enhancing the level of carotenoids in seeds, especially at enhancing β-carotene.

4. DISCUSSION

In *Arabidopsis*, *AtmiR156b* regulates plant growth and development, including the rate of leaf initiation (*27*) and transition from vegetative growth to reproductive growth (*4*, *6*, *7*) by negatively regulating the expression of 10 target *SPL* transcription factor genes (*1*−*3*, *27*). Suppression of *SPL* genes resulting

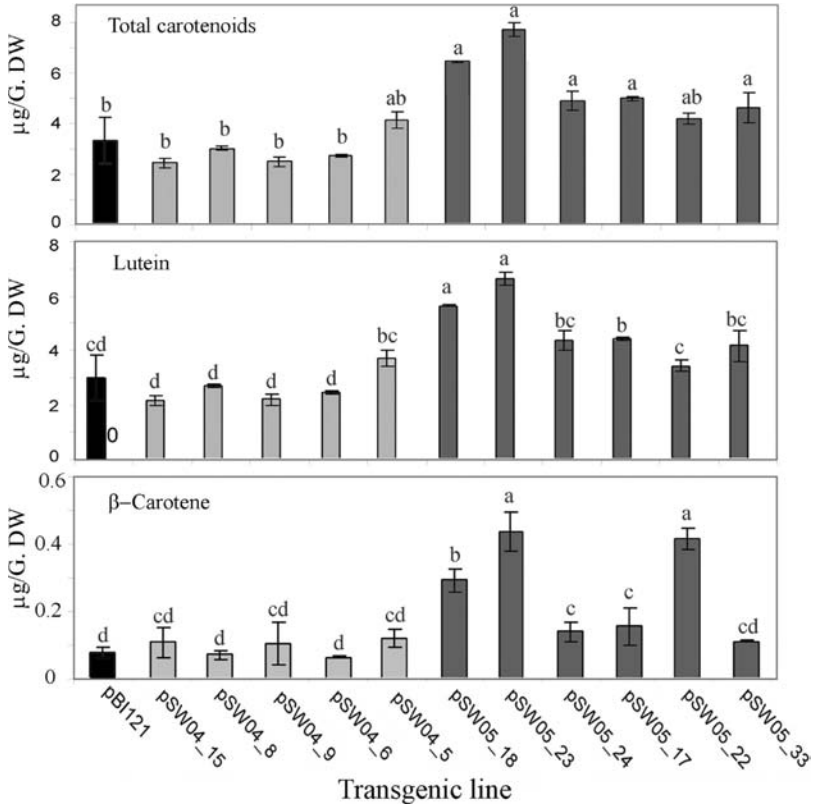

Figure 4. Enhanced seed lutein and β-carotene levels in *B. napus* constitutively expressing an *AtmiR156b* gene. Means with the same letter are statistically similar ($p \leq 0.05$). Error lines represent the standard deviation of the means of 3 replicates. pSW04 lines, *napin* promoter driven *AtmiR156b*; pSW05 lines, CaMV *35S* promoter driven *AtmiR156b*.

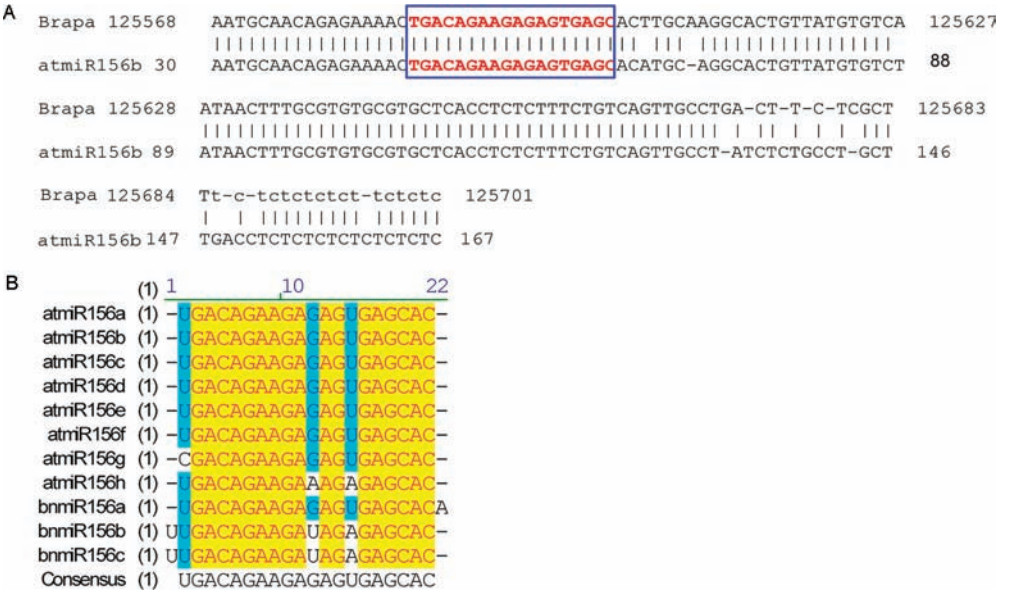

Figure 5. Alignment of miR156b precursors from *B. rapa* and *A. thaliana* (mature sequence highlighted in blue box) (**A**) and mature sequences of eight *miR156* genes from *Arabidopsis* and three genes from *B. napus* (**B**). Sequences highlighted in yellow are identical, while those highlighted in blue and white contain mismatches.

from *AtmiR156* overexpression has been well documented in *Arabidopsis*. Although, a similar suppression in transgenic *B. napus* overexpressing *AtmiR156b* could be deduced from the available literature, it would be interesting to study the functions of different *SPL* genes in *B. napus*, especially as they relate to the phenotypic changes observed in our study. Moreover, overexpression of *AtmiR156b* in *Arabidopsis* (*1*, *3*, *4*), rice (*28*), and maize (*29*), resulted in similar phenotypic alterations. Here, we show its effect on enhancing vegetative shoot branching and reproductive shoot branching when expressed in *B. napus* under the control of a constitutive promoter. This suggests an evolutionarily conserved role for *miR156* genes across diverse plant species.

A major new finding in our study is that 35S-driven *AtmiR156b* expression in *B. napus* caused increased levels of the seed carotenoids, lutein and β-carotene, which are strong antioxidants (*30*). Although a greater variability in seed carotenoid levels within individuals in T_1 transgenic lines than in T_0 lines would be expected due to transgene segregation in T_1 plants, the *AtmiR156b* induced seed carotenoid enhancement should be maintained. In silico sequence analysis of the whole *Arabidopsis* genome (TAIR 9) indicated that none of the known carotenoid pathway genes has a sequence complementary to the mature sequence of *AtmiR156b* (data not shown). Thus, *AtmiR156b* likely affects carotenoid pathways indirectly through one of the components of its gene regulation networks.

Recently, a group of carotenoid-derived metabolites, strigolactones, was determined to regulate branching in plants through a hormone regulatory network (*8*, *9*, *31*). Loss-of-function of carotenoid cleavage dioxygenase 7 and 8 results in an enhanced shoot branching phenotype in *Arabidopsis* (*30*). In addition, inactivation of histone methyltransferase SDG8 leads to low expression levels of a carotenoid isomerase gene, resulting in altered carotenoid composition in *Arabidopsis* and increased shoot branching (*32*). Since both seed carotenoids and branching are altered in *B. napus* lines expressing the *AtmiR156b* gene, it would be interesting to analyze these lines in the future for a better understanding of the mechanisms underlying the regulatory effects of the *AtmiR156b* gene on carotenoid metabolism in *B napus*.

Since flowering shoot branches were increased in pSW05 transgenic plants (in which *AtmiR156b* was expressed from a 35S promoter), we determined whether *AtmiR156b* had an impact on seed productivity. Our data suggested that in pSW05 plants increased reproductive branch number can reduce seed size, while overall seed yield is not affected. Reduced seed size could be related to the supply of fertilizer, which may not have been sufficiently optimized to support the extended growth and flowering period (> additional 2 months) that occurred with pSW05 plants. However, an increase in seed yield variability was also observed for pSW04 plants expressing *AtmiR156b* from the napin promoter, and these plants did not show increased branching. A better understanding of the functions of *AtmiR156* and its regulation of gene networks in *B. napus* is needed to pinpoint the specific gene regulation mechanism underlying seed weight variability and reduction.

Sequence analysis indicated that the mature sequences of *B. rapa BrmiR156b* (GenBank, Accession No. AC189375.2) and *Arabidopsis AtmiR156* are identical, while up to 90% identity is maintained between the premature *miR156b* sequences of these two species (**Figure 5A**). The mature *AtmiR156b* sequence also is identical to the mature *B. napus BnmiR156a* but has two mismatches when aligned to the mature sequence of *BnmiR156b* and *BnmiR256c* (http://www.mirbase.org/cgi/bin/mirna_summary.pl?fam= MIPF0000008) (**Figure 5B**). Plant microRNAs regulate target gene expression based on complementarity to their target genes, but 2−4 mismatches are tolerated (*2*). Transgenic *Arabidopsis* overexpressing either *AtmiR156b* or other *AtmiR156* genes, which have identical mature sequences, all exhibit the same morphological alterations including additional rosette leaves and delayed flowering time (*4*). Therefore, we suggest that *AtmiR156b* has the potential to function through *SPL* target gene expression regulation similarly when expressed in transgenic *B. napus* and *Arabidopsis*.

Seed-specific expression of the *AtmiR156b* gene in *B. napus* carrying the pSW04 construct had little or no impact on branching habit or seed carotenoid profiles, even though seed weight was affected in these plants. The seed likely is not a major functional

site of the *AtmiR156* gene since phenotypic alterations resulting from miR156 negative regulation in *Arabidopsis* are due to shoot-tip specific expression of some SPL genes (*26*). Moreover, the napin promoter is active only in the later stages of seed development (*33*).

In conclusion, the *AtmiR156b* was shown to function in *B. napus*. Constitutive expression increased vegetative and reproductive branching, often impacted seed weight, and increased seed carotenoid levels. In at least one line (pSW05-33), seed carotenoids were elevated without penalty on seed size or yield. Since the transgenic *B. napus* lines expressing *AtmiR156b* showed increased variability for each of those traits, it may be possible to identify lines with favorable seed carotenoid levels and few penalties, assuming other traditionally favorable characterisitics of seed quality (i.e., low glucosinolates and low erucic acid), disease resistance, and seedling vigor are present. Such lines would be welcome in a number of transgenic edible crops, including canola, since they would likely show enhanced human health attributes, antioxidant capacity, and oil with improved shelf life (*34*).

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LITERATURE CITED

(1) Schwab, R.; Palatnik, J. F.; Riester, M.; Schommer, C.; Schmid, M.; Weigel, D. Specific effects of microRNAs on the plant transcriptome. *Dev. Cell.* **2005**, *8* (4), 517–527.

(2) Rhoades, M. W.; Reinhart, B. J.; Lim, L. P.; Burge, C. B.; Bartel, B.; Bartel, D. P. Prediction of plant microRNA targets. *Cell* **2002**, *110* (4), 513–520.

(3) Gandikota, M.; Birkenbihl, R. P.; Hohmann, S.; Cardon, G. H.; Saedler, H.; Huijser, P. The miRNA156/157 recognition element in the 3′UTR of the *Arabidopsis* SBP box gene SPL3 prevents early flowering by translational inhibition in seedlings. *Plant J.* **2007**, *49* (4), 683–693.

(4) Wu, G.; Poethig, R. S. Temporal regulation of shoot development in *Arabidopsis thaliana* by miR156 and its target SPL3. *Development* **2006**, *133* (18), 3539–3547.

(5) Schwarz, S.; Grande, A. V.; Bujdoso, N.; Saedler, H.; Huijser, P. The microRNA regulated SBP-box genes *SPL9* and *SPL15* control shoot maturation in *Arabidopsis*. *Plant Mol. Biol.* **2008**, *67* (1−2), 183–195.

(6) Wu, G.; Park, M. Y.; Conway, S. R.; Wang, J. W.; Weigel, D.; Poethig., R. S. The sequential action of miR156 and miR172 regulates developmental timing in *Arabidopsis*. *Cell* **2009**, *138* (4), 750–759.

(7) Wang, J. W.; Czech, B.; Weigel, D. miR156-regulated SPL transcription factors define an endogenous flowering pathway in *Arabidopsis thaliana*. *Cell* **2009**, *138* (4), 738–749.

(8) Umehara, M.; Hanada, A.; Yoshida, S.; Akiyama, K.; Arite, T.; Takeda-Kamiya, N.; Magome, H.; Kamiya, Y.; Shirasu, K.; Yoneyama, K.; Kyozuka, J.; Yamaguchi, S. Inhibition of shoot branching by new terpenoid plant hormones. *Nature* **2008**, *455* (7210), 195–200.

(9) Gomez-Roldan, V.; Fermas, S.; Brewer, P. B.; Puech-Pagès, V.; Dun, E. A.; Pillot, J. P.; Letisse, F.; Matusova, R.; Danoun, S.; Portais, J. C.; Bouwmeester, H.; Bécard, G.; Beveridge, C. A.; Rameau, C.; Rochange, S. F. Strigolactone inhibition of shoot branching. *Nature* **2008**, *455* (7210), 189–194.

(10) Nesi, N.; Delourme, R.; Bregeon, M.; Falentin, C.; Renard, M. Genetic and molecular approaches to improve nutritional value of *Brassica napus* L. seed. *C. R. Biol.* **2008**, *331* (10), 763–771.

(11) Cooper, D. A. Carotenoids in health and disease: recent scientific evaluations, research recommendations and the consumer. *J. Nutr.* **2004**, *134* (1), 221S–224S.

(12) Rao, A. V.; Rao, L. G. Carotenoids and human health. *Pharmacol. Res.* **2007**, *55* (3), 207–216.

(13) Landrum, J. T.; Bone, R. A. Lutein, zeaxanthin, and the macular pigment. *Arch. Biochem. Biophys.* **2001**, *385* (1), 28–40.

(14) Landrum, J. T.; Bone, R. A. Dietary lutein and zeaxanthin: reducing the risk of macular degeneration. *Agro. Food Ind. Hi-Tech.* **2004**, *15* (6), 22–25.

(15) Howitt, C. A.; Pogson, B. J. Carotenoid accumulation and function in seed and non-green tissues. *Plant Cell Environ.* **2006**, *29* (3), 435–445.

(16) Giuliano, G.; Tavazza, R.; Diretto, G.; Beyer, P.; Taylor, M. A. Metabolic engineering of carotenoid biosynthesis in plants. *Trends Biotechnol.* **2008**, *26* (3), 139–145.

(17) Galpaz, N.; Ronen, G.; Khalfa, Z.; Zamir, D.; Hirschberg, J. A chromoplast-specific carotenoid biosynthesis revealed by cloning of the tomato *white-flower* locus. *Plant Cell* **2006**, *18* (8), 1947–1960.

(18) Lu, S.; Van Eck, J.; Zhou, X.; Lopez, A. B.; O'Halloran, D. M.; Cosman, K. M.; Conlin, B. J.; Paolillo, D. J.; Garvin, D. F.; Vrebalov, J.; Kochian, L. V.; Küpper, H.; Earle, E. D.; Cao, J.; Li, L. The cauliflower *Or* gene encodes a DnaJ cysteine-rich domain-containing protein that mediates high levels of beta-carotene accumulation. *Plant Cell* **2006**, *18* (12), 3594–3605.

(19) Davuluri, G. R.; van Tuinen, A.; Fraser, P. D.; Manfredonia, A.; Newman, R.; Burgess, D.; Brummell, D. A.; King, S. R.; Palys, J.; Uhlig, J.; Bramley, P. M.; Pennings, H. M. J.; Bowler, C. Fruit-specific RNAi-mediated suppression of *DET1* enhances carotenoid and flavonoid content in tomatoes. *Nat. Biotechnol.* **2005**, *23* (7), 890–895.

(20) Wei, S.; Li, X.; Gruber, M. Y.; Li, R.; Zhou, R.; Zebarjadi, A.; Hannoufa, A. RNAi-mediated suppression of DET1 alters the levels of carotenoids and sinapate esters in seeds of *Brassica napus*. *J. Agric. Food Chem.* **2009**, *24* (12), 5326–5333.

(21) Rask, L.; Ellerstrom, M.; Ezcurra, I.; Stalberg, K.; Wycliffe, P. Seed-specific regulation of the napin promoter in *Brassica napus*. *J. Plant Physiol.* **1998**, *152* (6), 595–599.

(22) Moloney, M. M.; Walker, J. M.; Sharma, K. K. High efficiency transformation of *Brassica napus*, using *Agrobacterium* vectors. *Plant Cell Rep.* **1989**, *8* (4), 238–242.

(23) Wei, S. Utility of tetracycline-inducible promoter system in transgenic *Catharanthus roseus* hairy roots. *Int. J. Plant Sci.* **2009**, *170* (7), 829–833.

(24) Shi, H.; Bressan, R. RNA Extraction. In *Arabidopsis Protocols*, 2nd ed.; Salinas, J., Jose, J., Sanchez-Serrano, J. J., Eds.; Humana Press: Totowa, NJ, 2006; pp 345−348.

(25) Yu, B.; Lydiate, D. J.; Schäfer, U. A.; Hannoufa, A. Characterization of a beta-carotene hydroxylase of *Adonis aestivalis* and its expression in *Arabidopsis thaliana*. *Planta* **2007**, *226* (1), 181–192.

(26) Nicot, N.; Hausman, J.-F.; Hoffmann, L.; Evers, D. Housekeeping gene selection for real-time RT-PCR normalization in potato during biotic and abiotic stress. *J. Exp. Bot.* **2005**, *56* (421), 2907–2914.

(27) Wang, J. W.; Schwab, R.; Czech, B.; Mica, E.; Weigel, D. Dual effects of miR156-targeted SPL genes and CYP78A5/KLUH on plastochron length and organ size in *Arabidopsis thaliana*. *Plant Cell* **2008**, *20* (5), 1231–1243.

(28) Xie, K.; Wu, C.; Xiong, L. Genomic organization, differential expression, and interaction of SQUAMOSA promoter-binding-like transcription factors and microRNA156 in rice. *Plant Physiol.* **2006**, *142* (1), 280–293.

(29) Chuck, G.; Cigan, A. M.; Saeteurn, K.; Hake, S. The heterochronic maize mutant Corngrass1 results from overexpression of a tandem microRNA. *Nat. Genet.* **2007**, *39* (4), 544–549.

(30) Iannone, A.; Rota, C.; Bergamini, S.; Tomasi, A.; Canfield, L. M. Antioxidant activity of carotenoids: an electron-spin resonance study on beta-carotene and lutein interaction with free radicals generated in a chemical system. *J. Biochem. Mol. Toxicol.* **1998**, *12* (5), 299–304.

(31) Leyser, O. The control of shoot branching: an example of plant information processing. *Plant Cell Environ*. **2009**, *32* (6), 694–703.
(32) Cazzonelli, C. I.; Cuttriss, A. J.; Cossetto, S. B.; Pye, W.; Crisp, P.; Whelan, J.; Finnegan, E. J.; Turnbull, C.; Pogson, B. J. Regulation of carotenoid composition and shoot branching in *Arabidopsis* by a chromatin modifying histone methyltransferase, SDG8. *Plant Cell* **2009**, *21* (1), 39–53.
(33) Fernandez, D. E.; Turner, F. R.; Crouch, M. L. *In situ* localisation of storage protein mRNAs in developing meristems of *Brassica napus* embryos. *Development* **1991**, *111* (2), 299–313.
(34) Khachatourians, G. G., McHughen, A., Nip, W. K., Scorza, R., Hui, Y. H., Eds. *Transgenic Plants and Crops*; Marcel Dekker Inc.: New York, 2002.

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