Enhanced oil accumulation in tobacco (*Nicotiana tabacum* L.) leaves by ectopic overexpression of *VgDGAT1a* for renewable production of biofuels

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To increase oil accumulation in the high-biomass vegetative organs of tobacco (*Nicotiana tabacum* L.) plants for renewable production of biofuels, *VgDGAT1a* isolated from developing seeds of *Vernonia galamensis* L. was ectopically overexpressed in tobacco leaves using a constitutive promoter. The transgenic tobacco leaves showed a 3.5–5.0-fold increase in oil content compared to the control, with a maximum increase of 9.2% (DW). The transgenic leaves also showed a substantial change in fatty acid composition, with significant enhancement of linoleic acid (18 : 2) and notable reduction of α-linolenic acid (18 : 3). The overexpression of *VgDGAT1a* exhibited no deleterious effect on other phenotypes in the tobacco plant. These results will facilitate development of a novel system for lipid metabolic engineering in vegetative organs of plants, as well as provide a platform for the production of biofuels using the vegetative organs of commercial non-food crops.

Keywords: Biofuels, metabolic engineering, plant oil, tobacco leaves, *VgDGAT1a* gene.

GIVEN the current concerns on a challenging set of interrelated global issues, including energy crises and depletion of natural resources, many researchers are developing alternative chemical and biofuel resources to meet market demands. Plant seed oils, which are primarily composed of triacylglycerols (TAGs), are produced in seeds and have a structure similar to that of petroleum hydrocarbons. TAGs derived from oilseeds, including canola, soybean and oil palm, can be used as an alternative feedstock for biofuel production[1]. However, a drawback to such use of seed oils is that it may affect the supply of vegetable oil for human consumption, since the availability of arable land is limited and the human population is increasing. In recent years, researchers have made progress in improving seed oil content by overexpressing genes that encode enzymes and transcription factors involved in TAG biosynthesis[2–6].

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The enzyme diacylglycerol acyltransferase (DGAT) has been extensively studied for its ability to modify seed oil content. It is a rate-limiting enzyme that drives the final acylation step in the synthesis of TAG. Therefore, DGAT is a promising target for increasing seed oil accumulation[7,8]. DGAT1, a member of the DGAT family, plays a major role in oil accumulation in the seeds of many plants[9,10]. Some studies have shown that overexpression of DGAT1 enhances seed oil content in transgenic plants[11–13].

Although many studies to increase oil production have specifically overexpressed DGAT1 in the seeds of oil crops, we constitutively overexpressed a *Vernonia galamensis* L. cDNA clone (*VgDGAT1a*) that encodes DGAT1 in the vegetative organs of tobacco, a high-biomass plant. In doing so, we aimed to engineer a non-food crop for TAG production in vegetative (non-seed) tissues such as leaves, stems, roots and storage organs, for use as biofuel feedstock. Tobacco has primarily been grown as a cash crop, and the leaves are smoked in cigarettes. However, it can generate up to 170 tonnes/ha of vegetative tissue when grown for biomass production alone[14]. Moreover, like hardwood trees, tobacco can be coppiced to stimulate re-sprouting from the stump after cutting, and thus multiple biomass harvests are possible in a single year. Although oil content of the green biomass is much lower than that of oil-crop seeds, tobacco represents an attractive and promising 'energy plant resource', and could also serve as a model for the utilization of other high-biomass plants for biofuel oil production because of its huge biomass and the possibilities of metabolic engineering.

Materials and methods

Biomaterial

Tobacco plants (*Nicotiana tabacum* L., *N. benthamiana*, and *N. tabacum* cv. *Samsun*-NN) were maintained in our laboratory. The seeds were surface-sterilized, plated on half-strength Murashige and Skoog (MS) solid medium

for germination and grown in a growth chamber. Seedlings were grown in a greenhouse under a 16 h light (25°C)/8 h dark (21°C) photoperiod.

Experimental methods

Cloning of a cDNA encoding VgDGAT1a from Vernonia: Total RNA was extracted from developing seeds of *V. galamensis* L. and full-length *Vernonia* cDNA of DGAT1a (GenBank accession no. EF653276) was amplified with the primers VgDGAT-F1(5′-CCGCTCGAGCGGATGG CGTTATTAGATACG-3′, in which the underlined sequence indicates the *Xho*I restriction site) and VgDGAT-R1 (5′-CACATCTAGATATTTGCTTTTCCCCTTTT-3′, in which the underlined sequence indicates the *Xba*I restriction site), and sequenced.

Transient expression in N. benthamiana: To study whether expression of *VgDGAT1a* in plant leaves improves leaf oil content, *VgDGAT1a* was transiently expressed in *N. benthamiana* leaves as previously described[15], with some minor modifications. *Agrobacterium tumefaciens* cultures containing the pBI 121 vector with *VgDGAT1a* were incubated until OD_{600} of the culture reached 0.6–0.8. The culture was centrifuged at 5000 *g* for 10 min, the precipitate was re-suspended in 50 ml of buffer (10 mmol/l $MgCl_2$, 10 mmol/l MES, 150 µmol/l acetosyringone, pH 5.8), and maintained at room temperature for 3 h. The re-suspended liquid was sampled using sterile needles and injected into the leaves of *N. benthamiana*. The plants were grown for five days under routine culture conditions. Then total lipids were extracted and determined by gas chromatography (GC)[16].

Construction of VgDGAT1a binary expression vectors and tobacco transformation: VgDGAT1a was cloned between the CaMV $35S^2$ promoter and the rbcS3′ terminator in the pCAMBIA 1301 vector (http://www.cambia.org/daisy/cambia/585.html, GenBank accession no. AF234297.1) to construct the $35S^2$-VgDGAT1a expression vector. Figure 1 shows schematic of the process. The $35S^2$-VgDGAT1a expression vector was transformed into leaf discs of eight-week-old sterile tobacco seedlings using *Agrobacterium*-mediated transformation[17,18]. Transgenic shoots were selected on hygromycin (50 mg/ml) and were rooted on half-strength MS medium containing hygromycin (50 mg/ml). The rooted shoots were transferred to potting mixture and hardened plants were established in a growth chamber.

PCR confirmation of transgenic plants and transgene expression: PCR and Southern blotting were used to detect the presence of *VgDGAT1a* in the T_0 transformed tobacco samples. Southern blotting was used to detect target gene integration according to a previously described method[19]. Seeds harvested from T_1 transgenic plants were placed on solid medium containing hygromycin for germination. Positive seedlings were then transferred to soil plots for growth to maturation. qRT-PCR and RT-PCR were used to detect the expression of *VgDGAT1a* in T_2 transgenic plants. The expression level of *VgDGAT1a* in different qRT-PCR samples was calculated using the $2^{-\Delta\Delta CT}$ method, as previously described[20].

Lipid-specific staining of cells: Tobacco leaf epidermis of mature six-week-old plants was stained with Nile red (10% (W/V) in acetone), a lipid-specific dye, and examined by fluorescence microscopy (Olympus, Japan). Lipid droplets appear as bright orange spherical granules in fluorescence microscopy.

Lipid analysis: To study the impact of *VgDGAT1a* overexpression on oil content and fatty acid composition, total lipids were extracted from leaves of six-week-old transgenic plants according to the method of Dahmer *et al.*[21]. Briefly, 10–20 mg of freeze-dried samples was placed in glass test tubes containing 3 ml H_2SO_4 : methanol (1 : 49, V/V), and heptadecanoic acid triglycerides were spiked to samples to estimate the oil content of the leaves. Samples were homogenized on ice and placed in a heating block (80°C) for 10 min, to reduce the liquid to 0.4 ml. Upon cooling, 1 ml hexane containing 0.01% (W/V) butylated hydroxytoluene was added to each tube. The samples were vortexed and then centrifuged at 1000 *g* for 5 min. The clarified supernatants (containing fatty acid methyl ester, FAME) were transferred to the gas chromatographic (GC) system (0.25 mm i.d. ×0.33 µm × 10 m FFAP column, FID detector; Agilent 7890A, USA) to determine the fatty acid composition and content of the lipid extracts. An inlet temperature of 220°C, a detector temperature of 250°C, and an inlet purge flow of 10 ml/min, activated at 1 min were used. The column was programmed with an initial temperature of 120°C for 1 min, then increased at 12°C/min to 210°C for 3 min, and further increased at 5°C/min to 235°C and held for 8 min. Helium was used as the carrier gas, with a flow rate of 10 ml/min.

Results

Effects of transient expression of VgDGAT1a on the oil content of N. benthamiana leaves

Total oil extracted from *N. benthamiana* leaves was determined by GC. The oil content in the infected leaves was significantly higher (~10%, dry weight, DW) than that of the uninfected leaves ($P < 0.001$). No significant changes were observed in leaf chlorophyll content (Figure 2). The results suggest that *VgDGAT1a* overexpression significantly increased the oil content of *N. benthamiana* leaves without adverse effects on photosynthesis. Thus, we can significantly enhance the oil content of

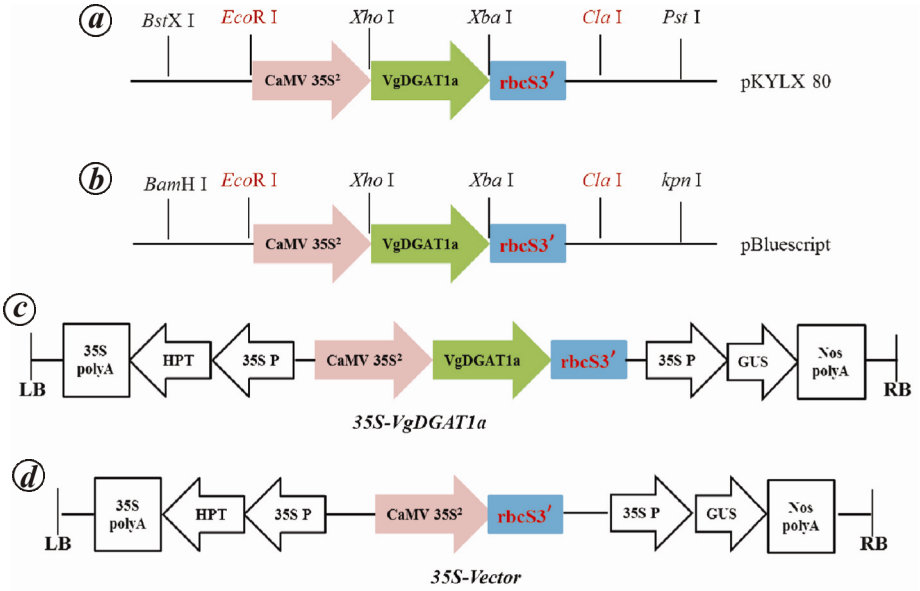

Figure 1. Schematic representation of *VgDGAT1a* expression constructs used for tobacco transformation. ***a***, *VgDGAT1a* subcloned in the pKYLX 80 vector. ***b***, *VgDGAT1a* expression cassette inserted into an intermediate vector pBluescript. ***c***, *VgDGAT1a* expression cassette inserted into the binary vector pCAMBIA 1301 to form the expression vector of *VgDGAT1a* driven by the $35S^2$ promoter ($35S^2$-VgDGAT1a). ***d***, Empty vector without the enzyme sequence used as vector control ($35S^2$-vector). P, Promoter; HPT, Hygromycin phosphotransferase; 35S Poly A, 35S poly(A) signal; Nos poly A, Nopaline synthase poly(A) signal and rbcS3′: Subunit E9 gene 3′-terminator.

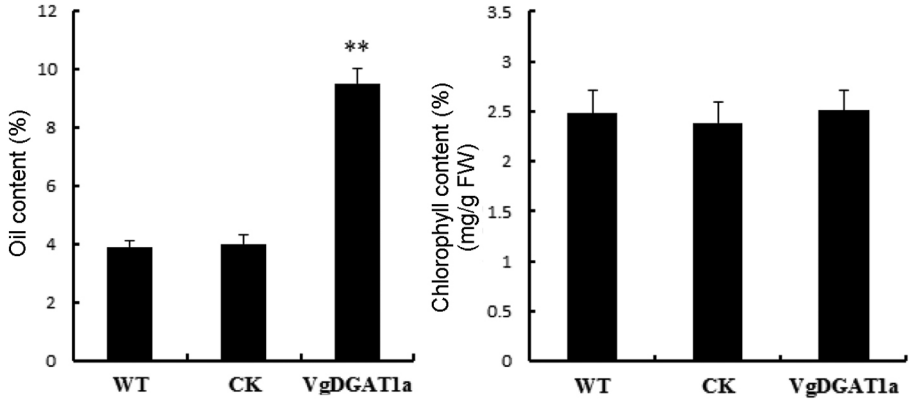

Figure 2. Effect of transient expression of *VgDGAT1a* on oil content (left) and chlorophyll content (right) of leaves of the *Nicotiana benthamiana*. WT, Wild-type lines; CK, Control vector lines; VgDGAT1a, *VgDGAT1a*-expressing lines. Significant at ** $P < 0.001$.

tobacco leaves by constitutive overexpression of *VgDGAT1a*.

Identification of transgenic VgDGAT1a tobacco lines

A total of 142 hygromycin-resistant transgenic tobacco plants from independent disc transformation were obtained in the T_0 generation. From these plants, 81 transgenic lines expressing *VgDGAT1a*, as detected by RT-PCR (Figure 3) and qRT-PCR, were obtained in the T_2 generation. qRT-PCR analysis of leaf samples indicated no statistically significant variation in *VgDGAT1a* expression between different transgenic lines ($P > 0.05$). The same results were found in RT-PCR with total RNA as loading control. Twenty lines expressing *VgDGAT1a*, from independent transformation events, were selected for subsequent analysis.

Analysis of lipid composition and content of transgenic tobacco leaves expressing VgDGAT1a

GC analysis showed that the leaf lipid content increased by 3.5–5.0-fold in the D1–D8 transgenic lines compared

to the wild-type leaves ($P < 0.001$). In particular, leaves of the D8 line showed a five-fold increase in lipid production, and in these leaves lipids accounted for 9.2% of leaf dry weight (Figure 4).

Fluorescence microscopy showed the presence of lipid droplets, which appeared as bright orange spherical granules in the cytoplasm of stained cells in transgenic plants (Figure 5). In contrast, wild-type lines showed very few lipid droplets. Taken together, these data demonstrate that, as expected, constitutive overexpression of *VgDGAT1a* significantly increased the lipid content in tobacco leaves.

In addition to increasing the lipid content of tobacco leaves, overexpression of *VgDGAT1a* altered the fatty acid composition, as determined by GC (Figure 6). The linoleic acid (18 : 2) content differed significantly between transgenic lines and wild-type plants ($P < 0.01$), with an increase of 41–61% (average = 51%). In contrast, the C18 : 3 fatty acid content decreased significantly in transgenic lines compared to wild-type plants, with the decrease ranging from 30% to 43% (average = 37%). The

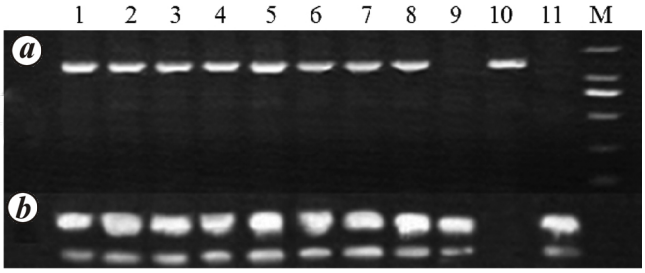

Figure 3. ***a***, RT-PCR analyses of *VgDGAT1a* expression in T2 transgenic tobacco. Lanes 1–8, *VgDGAT1a*-expressing lines; lane 9, Tobacco transformed with pCAMBIA1301 vector without the *VgDGAT1* gene; lane 10, PCR product of pCAMBIA1301 vector withthe *VgDGAT1a* gene as positive control; lane 11, Wild-type lines; lane 12, DL2000 DNA marker. RT-PCR forward primer: 5′-tgagctctccatggcgttattagatacg-3′, reverse primier:5′-cacatctagatatttgcttttccccttttt-3′, and full-length *VgDGAT1a* gene (1671 bp) was amplified, 30 cycles. ***b***, Ribosomal RNA bands indicate the relative total RNA loading in each lane (about 0.5 μg).

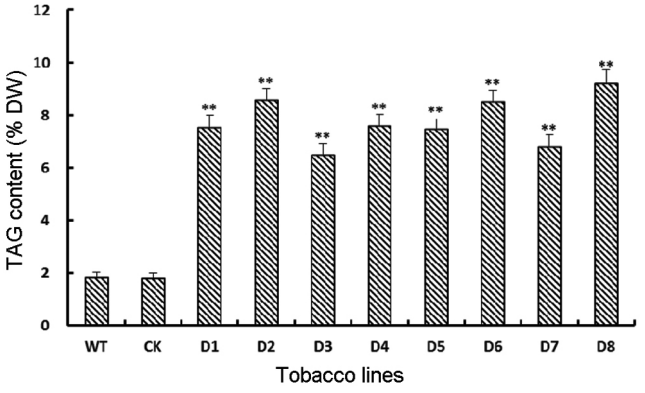

Figure 4. TAG content in *VgDGAT1a*-overexpressing tobacco leaves. WT, Wild-type lines; CK, Control vector lines and D1–D8, *VgDGAT1a*-expressing lines. Significant at $^{**}P < 0.001$.

C16 : 0 and C18 : 0 fatty acid contents decreased slightly compared to the wildtype, but the difference was not significant ($P > 0.05$).

Surprisingly, overexpression of *VgDGAT1a* caused production of C20 : 0 fatty acids, which were not found in leaf TAGs from wild-type plants, at a proportion of 3.63–4.33%. The relative proportions of unsaturated fatty acids and saturated fatty acids changed slightly, but the difference was not significant ($P > 0.05$).

Impact of VgDGAT1a overexpression on other phenotypes of tobacco plants

Although the lipid content increased significantly and fatty acid composition was substantially altered in *VgDGAT1a*-overexpressing transgenic tobacco leaves, no significant changes were observed in plant morphology (Figure 7), seed germination rate (Figure 8) and leaf chlorophyll content (Figure 9) compared to wild-type plants. These results indicate that *VgDGAT1a* overexpression significantly increased lipid content in tobacco leaves without adversely affecting plant growth and development.

Discussion

Application of metabolic engineering techniques to enhance accumulation of TAGs in vegetative tissues has recently been proposed as a promising strategy for increasing plant oil production. Andrianov *et al.*[22] overexpressed *Arabidopsis DGAT1* under the control of a strong rbcS promoter, resulting in an increase in TAG accumulation from 2.8% (DW) to 5.8% (DW) in wild-type tobacco leaves. Hanying *et al.*[23] reported that expression of *Arabidopsis DGAT1* under the control of the constitutive promoter CaMV 35S in tobacco increased the TAG content of leaves from 1.8% (DW) in wild-type plants to 7.2% (DW). Consistent with these results, we have shown that *VgDGAT1a* overexpression under control of the constitutive promoter CaMV 35S[2] leads to a 3.5–5.0-fold increase in oil accumulation in tobacco leaves, up to 9.2% (DW). *VgDGAT1a* was isolated from high-oil seeds of *V. galamensis*. Previous research indicated that *VgDGAT1a* has high enzymatic activity[8,24]. In soybean, overexpression of *VgDGAT1a* increased oil content by 5%, but overexpression of a fungal *DGAT2* only increased oil content by 1.5% (ref. 25). Our results show that *VgDGAT1a* can actively divert photosynthetic products to TAG biosynthesis, resulting in transgenic tobacco lines with a high level of TAG synthesis and accumulation.

DGAT1 catalyses downstream enzymatic processes in TAG synthesis. The increase in oil accumulation observed in the leaves of transgenic plants was solely due to the increased activity of DGAT1 overexpression by

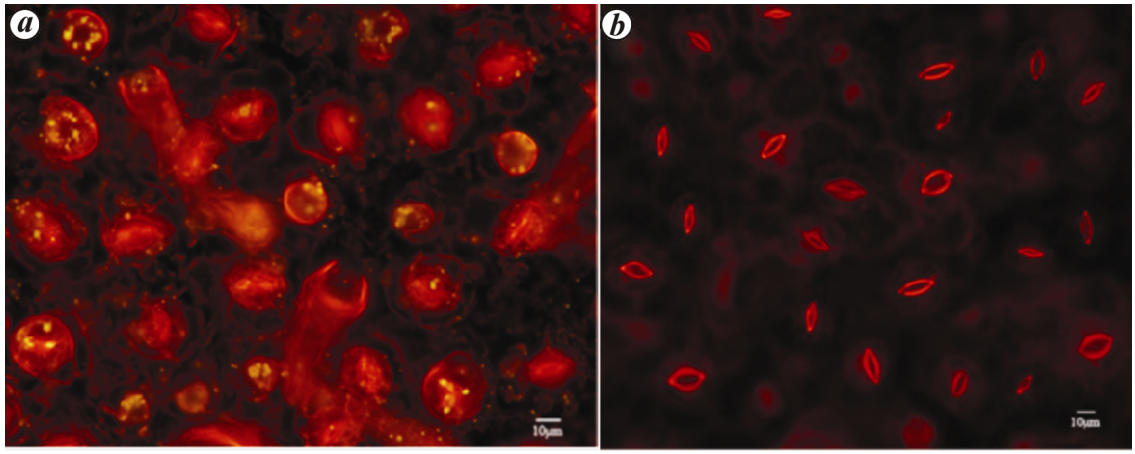

Figure 5. Image of Nile Red staining of leaves of wild-type and *VgDGAT1a*-expressing lines. ***a***, *VgDGAT1a*-expressing line and ***b***, wildtype. Neutral lipids are shown as yellow fluorescence. Bar = 10 μm.

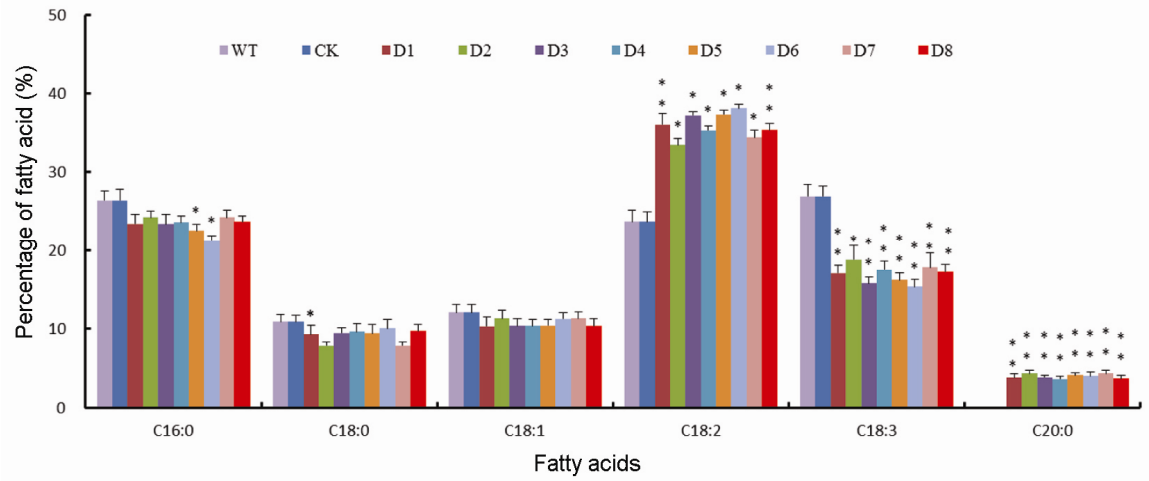

Figure 6. Fatty acid content in *VgDGAT1a*-overexpressing tobacco leaves. WT, Wild-type lines; CK, Control vector lines and D1–D8, *VgDGAT1*-expressing lines. Significant at $*P < 0.05$, $**P < 0.01$.

Figure 7. *VgDGAT1a*-overexpressing tobacco plant (***a***) and wild-type plant (***b***).

VgDGAT1a. Altering DGAT1 activity might also affect upstream steps in the fatty acid and TAG biosynthetic pathways, resulting in secondary metabolic or regulatory effects that contribute to increased TAG accumulation[26]. Enhancing DGAT1 activity may decrease the size of the acyl-CoA pool, thereby signalling a need for increased fatty acid synthesis[27].

Overexpression of *VgDGAT1a* in tobacco leaves not only resulted in accumulation of TAG, but also greatly altered the fatty acid composition of the TAG fraction. Linoleic acid increased significantly, accompanied by a reduction in linolenic acid in transgenic lines. No major changes were observed in palmitic acid (16 : 0), stearic acid (18 : 0) and oleic acid (18 : 1) compared with wild-type. Although the mechanism underlying the observed shifts in fatty acid composition remains unclear, our results are consistent with previous reports that DGAT1 overexpression results in a drastic shift in fatty acid composition in tobacco leaves[22]. Upregulation of TAG

biosynthesis pathways may result in more monounsaturated fatty acids entering the Kennedy pathway directly, making them largely inaccessible for further modification, such as subsequent desaturation[19].

Notably, arachic acid (20 : 0), which was not found in the green tissues of the wild-type, was detected in transgenic tobacco leaves. Arachic acid is known to show slight accumulation in the seeds of tobacco[28], but here overexpression of *VgDGAT1a* caused production of C20 : 0 in transgenic leaves, indicating that the function of *VgDGAT1a* in transgenic leaves is equivalent to that in seeds.

Several studies have shown that proteins, lipids and other nutrients stored in senescing leaves are broken down into small molecules and transported to seeds and other storage organs. The enriched lipids in transgenic tobacco leaves may be subject to this process during senescence, which is likely to affect the oil content of tobacco leaves. However, in production, the ability of tobacco stems to regenerate after harvest could be leveraged, and leaves/stems of tobacco with high oil content could be harvested to avoid leaf-oil degradation during ageing, without reducing the biomass obtained. In addition, inhibiting expression of lipid degradation genes using inducible promoters may increase lipid production[29].

Finally, although overexpression of *VgDGAT1a* in tobacco leaves resulted in the accumulation of TAG and altered the fatty acid composition of the TAG fraction, it did not significantly alter seed germination rates or other phenotypic characteristics of transgenic plants (Figures 8 and 9) compared to the wild-type. These data indicate that constitutive expression of genes that are specifically expressed in seeds in wild-type plants can result in lipid accumulation in vegetative organs. Recently, metabolic engineering has been used to produce tobacco lines with leaf-oil contents enriched in particular fatty acids[30]. These results suggest that additional gene stacking, construct optimization and further refinement of the current metabolic engineering strategies have the potential to yield unprecedented levels of storage lipids in non-seed tissues of a high biomass crop, and that fatty acid profiles could be simultaneously tailored to meet specific market needs, including biofuel, food, feed and biomaterials.

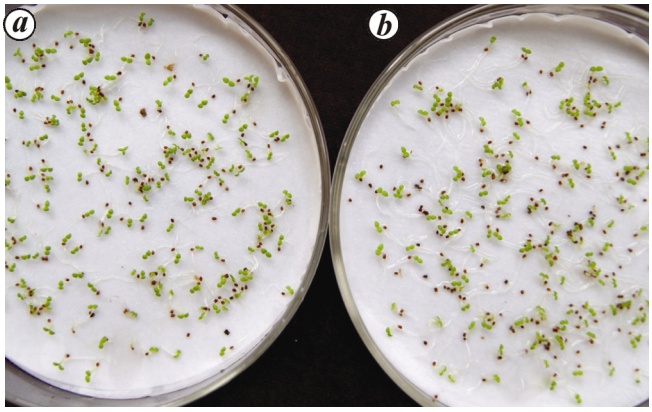

Figure 8. Germination of *VgDGAT1a*-transgenic (***a***) and wild-type (***b***) tobacco seeds.

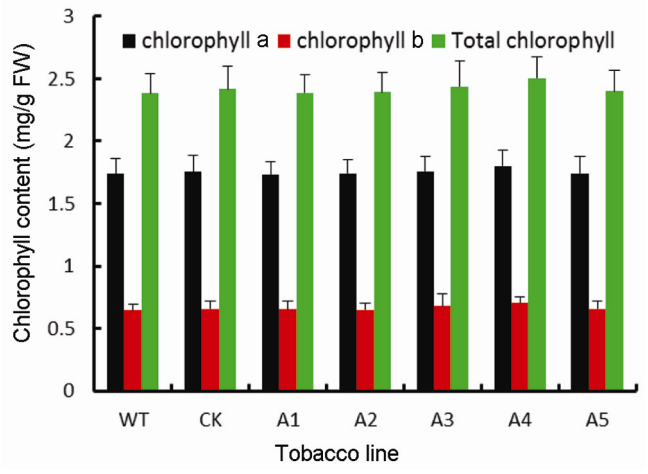

Figure 9. Leaf chlorophyll content of *VgDGAT1a*-transgenic and control leaves. WT, Non-transformed wild-type tobacco; CK, Empty vector–infected tobacco and D1–D5, Transgenic *VgDGAT1a* tobacco lines.

Conclusion

We have substantially enhanced the leaf lipid content in the high-biomass tobacco plant, accompanied by alteration of the fatty acid composition of the TAG fraction, by ectopic overexpression of *VgDGAT1a* from *V. galamensis*. This illustrates the great potential of upregulating lipid biosynthesis pathways in non-seed tissues. The present findings provide novel system for commercial production of TAGs using the high-biomass vegetative organs of a non-food crop.

1. Barthole, G., Lepiniec, L., Rogowsky, P. M. and Baud, S., Controlling lipid accumulation in cereal grains. *Plant Sci.: Int. J. Exp. Plant Biol.*, 2012, **185–186**(4), 33–39.
2. Ohlrogge, J. B. and Jaworski, J. G., Regulation of fatty acid synthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1997, **4**, 109–136.
3. Baud, S. and Lepiniec, L., Physiological and developmental regulation of seed oil production. *Prog. Lipid Res.*, 2010, **3**, 235–249.
4. Guschina, I. A., Everard, J. D., Kinney, A. J., Quant, P. A. and Harwood, J. L., Studies on the regulation of lipid biosynthesis in plants: application of control analysis to soybean. *Biochim. Biophys. Acta*, 2014, **6**, 1488–1500.
5. Tajima, D. *et al.*, Wrinkled 1 (WRI1) homologs, AP2-type transcription factors involving master regulation of seed storage oil synthesis in castor bean (*Ricinus communis* L.). *Am. J. Plant Sci.*, 2013, **2**, 333–339.
6. Wu, X.-L., Liu, Z.-H., Hu, Z.-H. and Huang, R.-Z., BnWRI1 coordinates fatty acid biosynthesis and photosynthesis pathways during oil accumulation in rapeseed. *J. Int. Plant Biol.*, 2014, **6**, 582–593.

7. Li, R., Yu, K., Hatanaka, T. and Hildebrand, D. F., *Vernonia* DGATs increase accumulation of epoxy fatty acids in oil. *Plant Biotechnol. J.*, 2010, **2**, 184–195.
8. Yu, K., Li, R., Hatanaka, T. and Hildebrand, D., Cloning and functional analysis of two type 1 diacylglycerol acyltransferases from *Vernonia galamensis*. *Phytochemistry*, 2008, **5**, 1119–1127.
9. Li, R., Yu, K. and Hildebrand, D. F., DGAT1, DGAT2 and PDAT expression in seeds and other tissues of epoxy and hydroxy fatty acid accumulating plants. *Lipids*, 2010, **45**, 145–157.
10. Zhang, F.-Y., Yang, M.-F. and Xu, Y.-N., Silencing of DGAT1 in tobacco causes a reduction in seed oil content. *Plant Sci.*, 2005, **4**, 689–694.
11. Jako, C. *et al.*, Seed-specific overexpression of an *Arabidopsis* cDNA encoding a diacylglycerol acyltransferase enhances seed oil content and seed weight. *Plant Physiol. Biochem.: PPB/Soc. Fran. Phys. Veg.*, 2001, **126**, 861–874.
12. Weselake, R. J. *et al.*, Metabolic control analysis is helpful for informed genetic manipulation of oilseed rape (*Brassica napus*) to increase seed oil content. *J. Exp. Bot.*, 2008, **59**, 3543–3549.
13. Shao, S. and Hegde, R. S., Membrane protein insertion at the endoplasmic reticulum. *Annu. Rev. Cell. Dev. Biol.*, 2011, **27**, 25–56.
14. Cramer, C. L. *et al.*, Bioproduction of human enzymes in transgenic tobacco. *Ann. N.Y. Acad. Sci.*, 1996, **25**, 62–71.
15. Voinnet, O., Rivas, S., Mestre, P. and Baulcombe, D., An enhanced transient expression system in plants based on suppression of gene silencing by the p19 protein of tomato bushy stunt virus. *Plant J.: Cell Mol. Biol.*, 2003, **5**, 949–956.
16. Youa, C.-X. *et al.*, Chemical constituents and biological activities of the Purple Perilla essential oil against *Lasioderma serricorne*. *Ind. Crops Prod.*, 2014, **61**, 331–337.
17. Grayburn, W. S., Collins, G. B. and Hildebrand, D. F., Fatty acid alteration by a Δ9 desaturase in transgenic tobacco tissue. *Bio/Technol.*, 1992, **6**, 675–678.
18. Bouvier-Navé, P., Benveniste, P., Oelkers, P., Sturley, S. L. and Schaller, H., Expression in yeast and tobacco of plant cDNAs encoding acyl CoA: diacylglycerol acyltransferase. *Eur. J. Biochem.*, 2000, **1**, 85–96.
19. Vanhercke, T., El Tahchy, A., Shrestha, P., Zhou, X. R., Singh, S. P. and Petrie, J. R., Synergistic effect of WRI1 and DGAT1 coexpression on triacylglycerol biosynthesis in plants. *FEBS Lett.*, 2013, **4**, 364–369.
20. Livak, K. J. and Schmittgen, T. D., Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, 2001, **4**, 402–408.
21. Dahmer, M. L., Fleming, P. D., Collins, G. B. and Hildebrand, D. F., A rapid screening technique for determining the lipid composition of soybean seeds. *J. Am. Oil Chem. Soc.*, 1989, **4**, 543–548.
22. Andrianov, V. *et al.*, Tobacco as a production platform for biofuel: overexpression of *Arabidopsis DGAT* and *LEC2* genes increases accumulation and shifts the composition of lipids in green biomass. *Plant Biotechnol. J.*, 2010, **3**, 277–287.
23. Hanying, W., Chao, L., Minchun, L., Mingming, Z., Dan, G. and Yinong, X., Effects of monogalactoglycerolipid deficiency and diacylglycerol acyltransferase overexpression on oil accumulation in transgenic tobacco. *Plant Mol. Biol. Rep.*, 2013, **5**, 1077–1088.
24. Li, R., Yua, K., Wu, Y., Tateno, M., Hatanaka, T. and Hildebrand, D. F., *Vernonia* DGATs can complement the disrupted oil and protein metabolism in epoxygenase-expressing soybean seeds. *Metab. Eng.*, 2012, **1**, 29–38.
25. Lardizabal, K. *et al.*, Expression of *Umbelopsis ramanniana* DGAT2A in seed increases oil in soybean. *Plant Physiol.*, 2008, **148**, 89–96.
26. Sharma, N. *et al.*, Transgenic increases in seed oil content are associated with the differential expression of novel *Brassica*-specific transcripts. *BMC Genomics*, 2008, **9**, 619.
27. Jako, C. *et al.*, Seed-specific over-expression of an *Arabidopsis* cDNA encoding a diacylglycerol acyltransferase enhances seed oil content and seed weight. *Plant Physiol. Biochem.*, 2001, **126**, 861–887.
28. Wen, O.-Y., Jianxiong, M. and Jingkai, D., Analytic study on the evaporable substances and fatty acids in aromatic (oriental) tobacco seeds. *Acta Tabacaria Sin.*, 1996, **2**, 51–55.
29. Slocombe, S. P. *et al.*, Oil accumulation in leaves directed by modification of fatty acid breakdown and lipid synthesis pathways. *Plant Biotechnol. J.*, 2009, **7**, 694–703.
30. Gao, C.-Y., Mao, X., Shang, H.-Q., Ji, X.-J. and Li, R.-Z., Ectopic overexpression of MucACP-Δ9 desaturase leads to ω-7 fatty acid accumulation in tobacco leaves. *Emir. J. Food Agric.*, 2016, **12**, 890–896.

ACKNOWLEDGEMENTS. This work was supported by the National Natural Science Foundation of China (Grant nos 30971806, 31201266 and 31401430), the state Ministry of Agriculture '948' Project of China (2014-Z39), Shanxi Province Key Projects of Coal-based Science and Technology of China (FT-2014-01), and Research Project Supported by Shanxi Scholarship Council of China (2015-064).

Received 28 December 2016; revised accepted 22 September 2017

doi: 10.18520/cs/v114/i06/1234-1240