NRC·CNRC

NRC Publications Archive
Archives des publications du CNRC

Stepwise metabolic engineering of docosatrienoic acid: an ω3 very long-chain polyunsaturated fatty acid with potential health benefits in Brassica carinata

Meesapyodsuk, Dauenpen; Sun, Kaiwen; Zhou, Rong; Thoms, Ken; Qiu, Xiao

This publication could be one of several versions: author's original, accepted manuscript or the publisher's version. / La version de cette publication peut être l'une des suivantes : la version prépublication de l'auteur, la version acceptée du manuscrit ou la version de l'éditeur.
For the publisher's version, please access the DOI link below./ Pour consulter la version de l'éditeur, utilisez le lien DOI ci-dessous.

Publisher's version / Version de l'éditeur:
https://doi.org/10.1111/pbi.13937
Plant Biotechnology Journal, 21, 1, pp. 8-10, 2022-09-28

NRC Publications Archive Record / Notice des Archives des publications du CNRC :
https://nrc-publications.canada.ca/eng/view/object/?id=2a5303fb-0285-48d5-884f-75c021439150
https://publications-cnrc.canada.ca/fra/voir/objet/?id=2a5303fb-0285-48d5-884f-75c021439150

Access and use of this website and the material on it are subject to the Terms and Conditions set forth at
https://nrc-publications.canada.ca/eng/copyright
READ THESE TERMS AND CONDITIONS CAREFULLY BEFORE USING THIS WEBSITE.

L'accès à ce site Web et l'utilisation de son contenu sont assujettis aux conditions présentées dans le site
https://publications-cnrc.canada.ca/fra/droits
LISEZ CES CONDITIONS ATTENTIVEMENT AVANT D'UTILISER CE SITE WEB.

Questions? Contact the NRC Publications Archive team at PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca. If you wish to email the authors directly, please see the first page of the publication for their contact information.

Vous avez des questions? Nous pouvons vous aider. Pour communiquer directement avec un auteur, consultez la première page de la revue dans laquelle son article a été publié afin de trouver ses coordonnées. Si vous n'arrivez pas à les repérer, communiquez avec nous à PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca.

National Research Council Canada | Conseil national de recherches Canada

Canada

Check for updates

Plant Biotechnology Journal
Open Access

Plant Biotechnology Journal (2023) **21**, pp. 8–10 doi: 10.1111/pbi.13937

Brief Communication

Stepwise metabolic engineering of docosatrienoic acid – an ω3 very long-chain polyunsaturated fatty acid with potential health benefits in *Brassica carinata*

Dauenpen Meesapyodsuk[1], Kaiwen Sun[2], Rong Zhou[3], Ken Thoms[4] and Xiao Qiu[1,2,*]

[1]*National Research Council Canada, Saskatoon, Canada*
[2]*Department of Food & Bioproduct Sciences, University of Saskatchewan, Saskatoon, Canada*
[3]*Saskatoon Research and Development Centre, AAFC, Saskatoon, Canada*
[4]*Saskatchewan Structural Science Centre, University of Saskatchewan, Saskatoon, Canada*

Received 20 July 2022;
revised 29 August 2022;
accepted 24 September 2022.
**Correspondence* (Tel 1-306-966-2181; fax 1-3069668898;
email xiao.qiu@usask.ca (X.Q))

Keywords: very long-chain polyunsaturated fatty acid, docosatrienoic acid, metabolic engineering, *Brassica carinata*.

Very long-chain polyunsaturated fatty acids (VLCPUFAs) are essential components of cell membranes and precursors for bioactive compounds regulating important physiological processes in humans and animals. Lack or imbalance of these fatty acids can lead to various physiological problems in humans such as immunological disorders, neurological conditions and cardiovascular diseases (Bazinet and Laye, 2014). The current market and transgenic plant production of VLCPUFAs is primarily focused on two ω3 VLCPUFAs, docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3), while other VLCPUFAs have been overlooked (Ganesh and Hettiarachchy, 2016; Napier *et al*., 2019; Qiu *et al*., 2020). Docosatrienoic acid (DTA, 22:3n-3) is an ω3 VLCPUFA with 22 carbons and three double bonds at 13, 16 and 19 positions, and was recently found to possess anti-inflammatory and antitumor properties comparable to DHA with potential nutraceutical and cosmetic uses (Chen *et al*., 2021). Biosynthesis of DTA follows the elongation and desaturation pathways of ω6 and ω3 polyunsaturated fatty acids (PUFAs; Figure 1a). In the ω3 pathway, α-linolenic acid (ALA, 18:3n-3) is elongated to eicosatrienoic acid (ETA, 20:3n-3) which is then elongated again to DTA (22:3n-3) by a single ELO type elongase (EhELO1) (Meesapyodsuk *et al*., 2018). In the ω6 pathway, linoleic acid (LA, 18:2n-6) is elongated to eicosadienoic acid (EDA, 20:2n-6) and elongated again to docosadienoic acid (DDA, 22:2n-6) by the same elongase. In addition, LA can be desaturated to ALA by a 18C-PUFA ω3 desaturase (CpDesX) while EDA can also be desaturated to ETA by a VLCPUFA ω3 desaturase (PiO3). Both desaturated products, ALA and ETA, can then be elongated to DTA by EhELO1.

To produce DTA in oilseed crop *B. carinata*, a dedicated oilseed crop in Canada for specialty oil, four constructs were made expressing the elongase and desaturases following the biosynthetic pathway (Figure 1b). Besides the expression cassettes with genes in the biosynthetic pathway, all constructs carried a herbicide phosphinothricin resistant gene under the control of a constitutive promoter for transformant selection (Meesapyodsuk *et al*., 2018). The first construct (Bc-1) expresses a single elongase EhELO1 from plant *Eranthis hyemalis* that can elongate a wide range of PUFAs, which has been reported previously (Meesapyodsuk *et al*., 2018). The second construct (Bc-2) expresses two genes encoding EhELO1 and CpDesX. CpDesX is an ω3 desaturase from fungus *Claviceps purpurea* with regioselectivity towards ω6-18C-PUFAs that can effectively convert LA to ALA (Meesapyodsuk *et al*., 2007). The third construct (Bc-3) expresses three genes coding for EhELO1, CpDesX and EhLPAAT2. EhLPAAT2 is an endoplasmic lysophosphatidic acid acyltransferase from *E. hyemalis* that can incorporate VLCPUFAs into the *sn-2* position of triacylglycerols, as *B. carinata* lacks the capacity (Meesapyodsuk *et al*., 2021). The fourth construct (Bc-4) expresses EhELO1, CpDesX, EhLPAAT2 and PiO3. PiO3 is another ω3 desaturase from fungus *Pythium irregulare* that can convert ω6-VLCPUFAs to ω3-VLCPUFAs particularly for ω6-20C-VLCPUFAs (Cheng *et al*., 2010). Each of these genes in the four constructs was under the control of a seed-specific promoter, *napin* or *conlinin*, and an octopine synthase (OCS) terminator.

The three new constructs were introduced into a low erucic acid breeding line through a Agrobacterium-mediated transformation approach using petioles as explants (Cheng *et al*., 2010). Transgenic plants selected with phosphinothricin and genomic DNA PCR were grown in growth cabinets at 22 °C under a 16-h-light (120 $\mu Em^{-2}/s$)/8-h-dark photoperiod. Fatty acid analysis of transgenic *B. carinata* by GC-FID expressing the first construct Bc-1 with *EhELO1* alone has been reported previously (Meesapyodsuk *et al*., 2018), producing several new VLCPUFAs such as EDA, ETA, DDA and DTA. Among them, DDA was the most abundant, followed by EDA, DTA and ETA. Transgenic *B. carinata* expressing the second construct Bc-2 with two genes *EhELO1* and *CpDesX* produced a similar fatty acid profile as Bc-1; however, the abundance of fatty acids varied significantly. Particularly, the abundance of LA and ALA was the reverse of each other in Bc-1 and Bc-2. This was due to the desaturation activity of CpDesX on LA, giving rise to ALA. The high level of ALA for elongation by EhELO1 resulted in the higher level of DTA than DDA in Bc-2 transgenic plants. Transgenic *B. carinata* expressing the third construct Bc-3 produced a similar fatty acid profile as Bc-2, but the amount of DTA was further increased, which was due to the pulling activity of EhLPAAT2 in the incorporation of VLCPUFAs into the *sn-2* position of TAGs (Meesapyodsuk *et al*., 2021). Transgenic plants expressing the four gene construct Bc-4 produced a similar fatty acid profile as Bc-3, but with a higher level of ETA than EDA due to the desaturation from EDA to ETA catalysed by PiO3. The higher level of ETA for elongation by

© 2022 National Research Council Canada and The Authors. *Plant Biotechnology Journal* published by Society for Experimental Biology and The Association of Applied Biologists and John Wiley & Sons Ltd. Reproduced with the permission of the Minister of Innovation, Science, and Economic Development.
This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

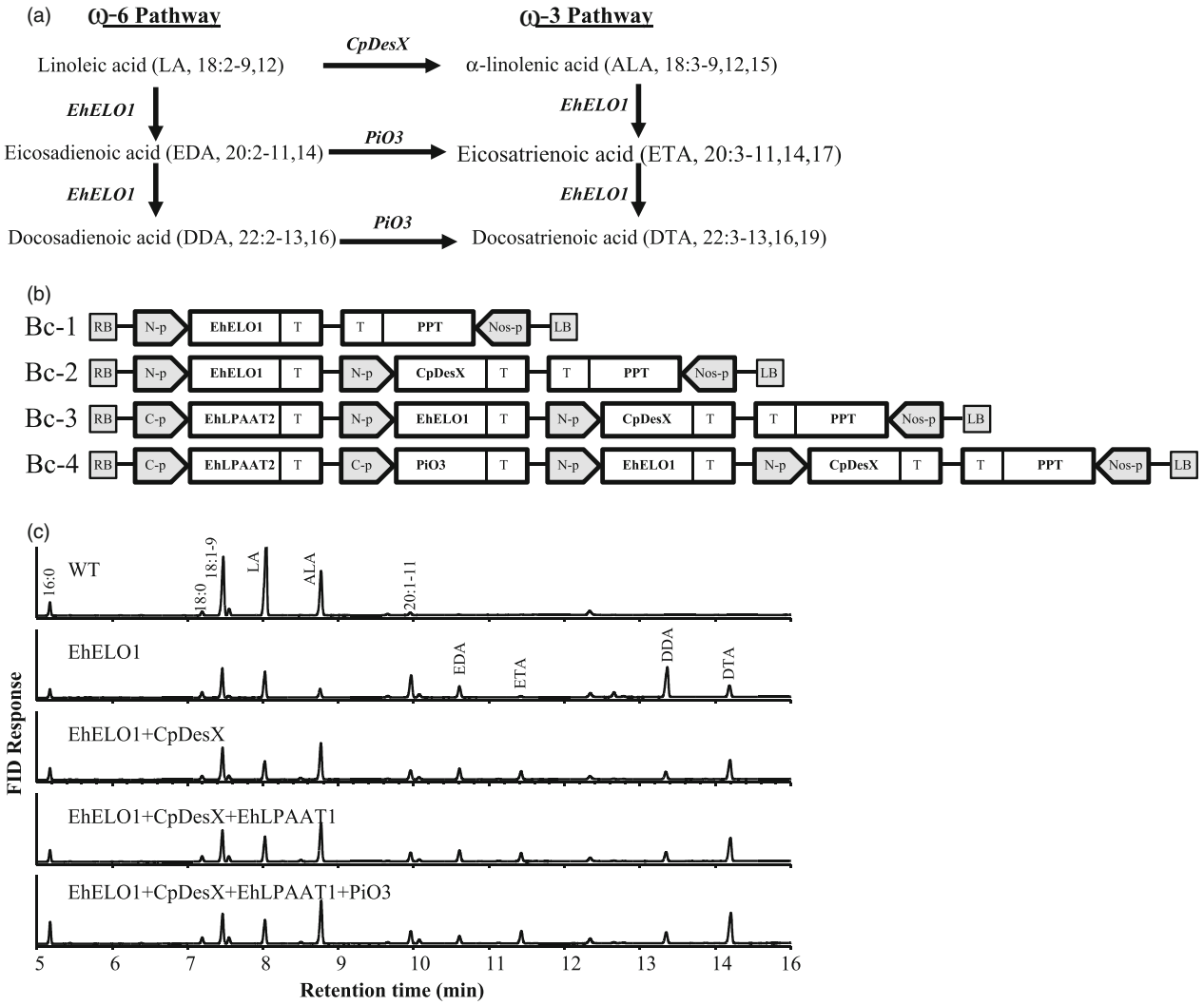

FIGURE 1 Stepwise metabolic engineering of DTA in *Brassica carinata*. (a) Biosynthetic pathway of DTA. (b) Simplified maps of the binary vectors used for *B. carinata* transformation. N-p: napin promoter; C-p: conlinin promoter; Nos-p: NOS promoter; T: ocs terminator; PPT: gene for phosphinothricin N-acetyltransferase. (c) GC analysis of T1 transgenic seeds expressing one-, two-, three- and four-gene constructs.

EhELO1 resulted in a further increase of DTA in Bc-4 (Figure 1c, Table S1). One elite line of Bc-4 transgenic plants was selected for propagation to the next generations. Fatty acid analysis of transgenic seeds from the three generations showed that the amount of DTA was slightly increased over these generations. DTA was in a range from 16% to 20% along with DDA in a range of 4%–6% over the three generations. The amount of DTA was more than three times that of DDA, accounting for 20% on average in the T3 transgenic plants (Table S2).

MALDI-TOF/MS was then utilized to profile the TAG species (Hong *et al.*, 2002) in the seeds of T3 transgenic plants of selected elite lines with a single *EhELO1* and four genes (*EhELO1*+*CpDesX*+*EhLPAAT2*+*PiO3*). Untransformed *B. carinata* produced three major fatty acids, 18:1 (oleic acid), LA and ALA where the major molecular species of TAGs were ALA/LA/18:1, LA/LA/LA, LA/LA/18:1, ALA/LA/LA and LA/18:1/18:1 according to the relative abundance. Transgenic *B. carinata* expressing EhELO1 alone produced many new TAG species such as DDA/ALA/EDA (or DTA/LA/EDA), DDA/18:1/18:1 (or 20:1/LA/20:1), DDA/LA/DDA (or DDA/18:1/DTA), and DDA/18:1/16:0 (or 20:1/LA/18:0). Those were also among the most abundant TAG species. As compared to those in wild type, molecular weights of TAGs in the *EhELO1* transgenics were shifted up by 2 to 8 carbons, indicating one to two VLCPUFAs such as DDA, DTA and EDA were incorporated into TAGs. Transgenic *B. carinata* expressing the four genes (EhELO1+CpDesX+EhLPAAT2+PiO3) further produced many new TAG species with three VLCPUFAs such as DTA/DTA/24:3 and DTA/DTA/DDA. Among all TAG profiles produced, those TAG species with three VLCPUFAs were the most abundant, followed by those with one or two VLCPUFAs such as DTA/DDA/ALA, DTA/ALA/ETA, DTA/18:1/LA, DDA/ALA/16:0. In TAGs with one or two VLCPUFAs, the shift of the molecular weights down by 2 to 4 daltons, as compared to those in EhELO1 transgenic seeds, indicated an addition of one or two double bonds in acylated fatty acids (Figure S1).

In summary, ω3-VLCPUFA DTA possesses potential health benefits, but the source for this fatty acid does not exist in nature. This study employed a stepwise strategy to engineer the biosynthetic pathway in *B. carinata*; about 20% DTA of the total fatty acids was produced in the transgenics. No obvious

© 2022 National Research Council Canada and The Authors. *Plant Biotechnology Journal* published by Society for Experimental Biology and The Association of Applied Biologists and John Wiley & Sons Ltd. Reproduced with the permission of the Minister of Innovation, Science, and Economic Development., **21**, 8–10

14677652, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/pbi.13937 by Cochrane Canada Provision, Wiley Online Library on [22/08/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

phenotypic changes such as seed germination, seedling growth and development were observed in the plants. The amount of DTA in seeds remained stable over three generations where DTA was mainly placed in TAG species with two or three VLCPUFAs. Efficient and sustainable production of DTA in the oilseed crop provides an opportunity for this fatty acid to be used in cosmetics, foods and feeds.

Acknowledgement

We would like to thank Shengjian Ye, Nagamani Datla and Yi Chen for technical assistance in plant transformation and growth, and Dr. Robert Chapman for the initial discussion of project. This is National Research Council of Canada publication number 58317.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

XQ and DM perceived the project and designed the experiments, DM conducted primary experiments, KS did the plant and fatty acid analysis, RZ analysed the oil content, KT did the MALDI-TOF/MS analysis of TAGs, and XQ and DM wrote the paper.

References

Bazinet, R.P. and Laye, S. (2014) Polyunsaturated fatty acids and their metabolites in brain function and disease. *Nat. Rev. Neurosci.* **15**, 771–785.

Chen, Y., Qiu, X. and Yang, J. (2021) Comparing the in vitro antitumor, antioxidant and anti-inflammatory activities between two new very long chain polyunsaturated fatty acids, docosadienoic acid (DDA) and docosatrienoic acid (DTA), and docosahexaenoic acid (DHA). *Nutr. Cancer*, **73**, 1697–1707.

Cheng, B., Wu, G., Vrinten, P., Falk, K., Bauer, J. and Qiu, X. (2010) Towards the production of high levels of eicosapentaenoic acid in transgenic plants: the effects of different host species, genes and promoters. *Transgenic Res.* **19**, 221–229.

Ganesh, V. and Hettiarachchy, N.S. (2016) A Review: supplementation of foods with essential fatty acids-can it turn a breeze without further ado? *Crit. Rev. Food Sci. Nutr.* **56**, 1417–1427.

Hong, H., Datla, N., Reed, D.W., Covello, P.S., MacKenzie, S.L. and Qiu, X. (2002) High-level production of γ-linolenic acid in *Brassica juncea* using a Δ6 Desaturase from *Pythium irregulare*. *Plant Physiol.* **129**, 354–362.

Meesapyodsuk, D., Reed, D.W., Covello, P.S. and Qiu, X. (2007) Primary structure, regioselectivity, and evolution of the membrane-bound fatty acid desaturases of *Claviceps purpurea*. *J. Biol. Chem.* **282**, 20191–20199.

Meesapyodsuk, D., Ye, S., Chen, Y., Chen, Y., Chapman, R.G. and Qiu, X. (2018) An engineered oilseed crop produces oil enriched in two very long chain polyunsaturated fatty acids with potential health-promoting properties. *Metab. Eng.* **49**, 192–200.

Meesapyodsuk, D., Chen, Y., Ye, S., Chapman, R.G. and Qiu, X. (2021) Co-expressing *Eranthis hyemalis* lysophosphatidic acid acyltransferase 2 and elongase improves two very long chain polyunsaturated fatty acid production in *Brassica carinata*. *Metab. Eng. Commun.* **12**, e00171.

Napier, J.A., Olsen, R.E. and Tocher, D.R. (2019) Update on GM canola crops as novel sources of omega-3 fish oils. *Plant Biotechnol. J.* **17**, 703–705.

Qiu, X., Xie, X. and Meesapyodsuk, D. (2020) Molecular mechanisms for biosynthesis and assembly of nutritionally important very long chain polyunsaturated fatty acids in microorganisms. *Prog. Lipid Res.* **79**, 101047.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 TAG molecular species in *B. carinata* transgenic seeds expressing one- and four-genes.

Table S1 Fatty acid compositions of T1 *B. carinata* transgenic seeds expressing one-, two-, three- and four-gene constructs (weight %).

Table S2 Fatty acid compositions and oil contents of an elite line expressing the four genes over three generations (weight %).

© 2022 National Research Council Canada and The Authors. *Plant Biotechnology Journal* published by Society for Experimental Biology and The Association of Applied Biologists and John Wiley & Sons Ltd. Reproduced with the permission of the Minister of Innovation, Science, and Economic Development., **21**, 8–10

14677652, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/pbi.13937 by Cochrane Canada Provision, Wiley Online Library on [22/08/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License