Brief Communication

Engineering docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) in Brassica juncea

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Omega-3 long-chain polyunsaturated fatty acids (ω3 LC-PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are important for human health. Suboptimal levels of ω3 LC-PUFAs are associated with increased risk of several diseases (Ghasemi Fard et al., 2019). Docosapentaenoic acid (ω3-DPA, C22:5) is a rare LC-PUFA but of special interest because of its unique properties (Drouin and Legrand, 2019; Kaur et al., 2013). Multiple studies showed direct effects of DPA on inflammation, improved plasma lipid profile and cognitive function (Ghasemi Fard and Cameron-Smith, 2021). The principal sources of DPA are wild oceanic fish species. However, DPA is not currently available in sufficient quantities for commercial production. An inexpensive and sustainable supply of this important ω3 fatty acid is highly desirable to conduct large-scale human intervention studies to examine the role of ω3-DPA in relation to optimal health.

Significant efforts to engineer the production of ω3 LC-PUFAs in oilseed crops have been attempted recently. Two distinctive approaches have been used to produce ARA, EPA and DHA in seed oils comparable to the levels of wild fish oil (Petrie et al., 2020; Usher et al., 2017; Walsh et al., 2016). These include the anaerobic polyketide synthase system and the aerobic desaturase pathway of LC-PUFA biosynthesis. The aerobic pathway involves sequential desaturation and elongation steps (Robert et al., 2005, Figure 1). Introduction of additional Δ12-desaturase (Δ12-Des) and ω3-desaturase (ω3-Des) in oil crop enhanced the DHA level (Petrie et al., 2020). However, there has been no attempt to produce DPA in higher plants. We introduced the aerobic LC-PUFA biosynthesis pathway into Brassica juncea and produced high levels of both DHA and DPA, the first successful production of DPA in a crop. The level of DPA was two to three times higher than the highest found in fish oil, providing a scalable platform for efficient DPA production. We also report the first successful production of DHA to DPA in 1:1 ratio in B. juncea seed oil.

Brassica juncea was transformed with the binary vector GA7_ModB (Figure 1a) used previously to develop DHA canola (Petrie et al., 2020). Full T-DNA insertion from this vector produces DHA, while an incomplete T-DNA insertion could lead to the accumulation of intermediates, including DPA (Figure 1b). Seed fatty acid composition was analysed by gas chromatography (Zhou and Singh, 2013). DPA positional distribution on triacylglycerol was determined as previously described (Petrie et al., 2014). Seed oil content was verified by NMR using an MQC benchtop analyser (Oxford Instruments) following the manufacture’s instruction.

Among 21 independent transgenic B. juncea lines, DHA levels in pooled T1 seeds ranged from 0% to 6.6% of total fatty acids. Interestingly, Line 4 had a substantial level of DPA (3.7%), and 6.6% DHA. Single T1 seed analysis of Line 4 showed a DPA content from 0.3% to 16.1% and DHA from 0 to 17.9%. Eight of 30 single T1 seeds contained 2.5–16.1% DPA but no DHA. Line 4 was then further analysed for fatty acid composition in half cotyledons of 48 germinating seeds. A range of 3.8–18.1% DPA was observed in half cotyledons of 11 T1 seeds, without any DHA, while others contained various levels of DHA. This suggested there was a segregation of multiple T-DNA insertions in T1 seeds leading to either DHA or DPA accumulation. Nineteen plants with either high DHA or high DPA without DHA were established. Fourteen of these plants had 3.6–17.2% DHA in T2 seeds. One progeny with 17.2% DHA was designated as BjDHA-4-17 and advanced to T4 seeds by selfing. The DHA level in T4 seeds remained 17% (Figure 1c). The other five T1 plants contained 4.2–12.5% DPA with no DHA in T2 seeds. These were designated BjDPA-4-13, BjDPA-4-19, BjDPA-4-25, BjDPA-4-34 and BjDPA-4-39, potentially containing truncated inserts without a functional Δ4-desaturase gene (Δ4-Des). BjDPA-4-34 was advanced to T6 seeds which contained 12 ± 1.3% DPA.

Line BjDHA-4-17 (17.2% DHA) was crossed with line BjDPA-4-19 (11.6% DPA), resulting in 68 F1 seeds. Half cotyledon analysis revealed that 11 F1 seeds contained both 4.1–6.0% DPA and 14.7–20.3% DHA. Pooled seed analysis of F2 seeds from these 11 F1 plants showed DPA levels ranging from 1.3% to 7.2% and DHA from 4.0% to 10.2%. Progeny 52, which had 7.2% DPA and 10.1% DHA in F2 seeds, was advanced to F2 plant. Pooled seed analysis of F3 seeds from 14 F2 plants showed variation in the amount of DHA, DPA and the sum of DHA+DPA, including six plants with an almost 1:1 DPA:DHA ratio (Figure 1c).

Sequencing of genomic DNA from the seedlings of BjDPA-4-34-2-8-7 (T2) showed there were three partial inserts containing the functional gene cassettes from GA7_ModB except for the ω3-
Des and Δ4-Des, leading to no conversion of DPA to DHA (Figure 1b). The function of the missing ω3D, converting C18:2 to C18:3, was complemented by the endogenous D15-desaturase.

Seed oil content remained same in T 5 (34.3 ± 2.1%) and T 6 (33.5 ± 2.0%) seeds derived from BjDPA-4 compared to the wild type (35.1 ± 2.1%) grown at the same time with no statistical difference. DPA was preferentially located at the sn-1/3 positions (91.6%) of the triacylglycerol molecules. Similar preferential distribution of DHA was previously reported (Petrie et al., 2020).

In this study, we explored the introduction of LC-PUFA biosynthesis pathway into B. juncea to produce DPA or DHA. Their levels were stable over four generations in BjDHA-4-17 and six generations in BjDPA-4-34. Although T-DNA truncations were observed and integration occurred at three different loci in the BjDPA-4 event, DPA levels were stable in both the glasshouse and field over several years and up to the T 6 generation. The oil from BjDPA lines has several unique features. It is relatively high in DPA, a highly beneficial ω3 LC-PUFA for dietary supplementation with increasing interest from the medical community (Kaur et al., 2013). In addition, the oil contained a high level of α-linolenic ALA (ca. ~20%, compared to 15% in WT B. juncea oil), contributing to an increased ω3:ω6 ratio, with concomitant health benefits. An almost 1:1 ratio of DHA to DPA was produced in F3 pooled seeds from the BjDHA × BjDPA crosses, with a total of ~17% DPA+DHA in the seed oil (Figure 1c). An oil with DHA and DPA in a 1:1 ratio may be an excellent source for promoting cardiovascular health.

This study demonstrates the production of 12% of DPA (two to three times higher than any other natural source) or 18% DHA in transgenic B. juncea, and the production of equal amounts of DHA and DPA in seed oil. Production of DPA in B. juncea is also more sustainable, removing the need to exploit ocean resources.

Figure 1  (a) Binary vector GA7_Mod-B used for this study (see Petrie et al., 2020 for detail); (b) LC-PUFA biosynthesis pathway leads to the production of DHA (with complete insert from the binary vector) or DPA (without Δ4-Des). (c) Fatty acid profile of BjDHA and BjDPA pooled seeds derived from Line 4, and their crossing progeny (n ≥ 6).

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Conflict of interest
The authors declare no conflict of interest.

Author contributions
SB, JRP, SPS and XRZ designed the experiments; SB, PS, YK and AL performed the experiments; SB, MDD, SPS and XRZ analysed data and wrote the manuscript.

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References
Drouin, G., Rioux, V. and Legrand, P. (2019) The n-3 docosapentaenoic acid (DPA): a new player in the n-3 long chain polyunsaturated fatty acid family. Biochimie, 159, 36–48.
Ghasemi Fard, S., Cameron-Smith, D. and Sinclair, A.J. (2021) n-3 Docosapentaenoic acid: the iceberg n-3 fatty acid. Curr. Opin. Clin. Nutr. Metab. Care, 24, 134–138.
Ghasemi Fard, S., Wang, F., Sinclair, A.J., Elliott, G. and Turchini, G.M. (2019) How does high DHA fish oil affect health? A systematic review of evidence. Crit. Rev. Food Sci. Nutr. 59, 1684–1727.
Kaur, G., Molero, J.C., Weisinger, H.S., Sinclair, A.J. (2013) Orally administered
[^1C]DPA and[^1C]DHA are metabolised differently to [^1C]EPA in rats. Br. J.
Nutr. 109, 441–448.

Petrie, J.R., Shrestha, P., Belide, S., Kennedy, Y., Lester, G., Liu, Q., Divi, U.K. et al. (2014) Metabolic engineering Camelina sativa with fish oil-like levels of
DHA. PLoS One, 9, e85061.

Petrie, J.R., Zhou, X.-R., Leonforte, A., McAllister, J., Shrestha, P. and Kennedy, Y. et al. (2020) Development of a Brassica napus (canola) crop containing fish
oil-like levels of DHA in the seed oil. Front. Plant Sci. 11, 727.

Robert, S.S., Singh, S.P., Zhou, X.-R., Petrie, J.R., Blackburn, S.I., Mansour, P.M.,
Nichols, P.D. et al. (2005) Metabolic engineering of Arabidopsis to produce
nutritionally important DHA in seed oil. Funct. Plant Biol. 32, 473–479.

Usher, S., Han, L., Haslam, R.P., Michaelson, L.V., Sturtevant, D., Aziz, M.,
Chapman, K.D. et al. (2017) Tailoring seed oil composition in the real world:
optimising omega-3 long chain polyunsaturated fatty acid accumulation in
transgenic Camelina sativa. Sci. Rep. 7, 6570.

Walsh, T.A., Bevan, S.A., Gachotte, D.J., Larsen, C.M., Moskal, W.A. and
Merlo, P.A.O. et al. (2016) Canola engineered with a microalgal polyketide
synthase-like system produces oil enriched in docosahexaenoic acid. Nat.
Biotechnol. 34, 881–887.

Zhou, X.-R., Singh, S.P. and Green, A.G. (2013) Characterisation of the FAD2
gene family from Hiptage benghalensis: a ricinoleic acid accumulating plant.
Phytochemistry, 92, 42–48.