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Datasheet for the decision of 7 September 2021

Case Number: T 1429/17 - 3.3.04

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Language of the proceedings: EN

Title of invention:
Synthesis of long-chain polyunsaturated fatty acids by recombinant cells

Patent Proprietor:
Commonwealth Scientific and Industrial Research Organisation

Opponent:
BASF SE

Headword:
Long-chain PUFA in plants/CSIRO

Relevant legal provisions:
EPC Art. 56, 87(1)
RPBA 2020 Art. 13(2)

Keyword:
Priority - (no)
Inventive step - Main and auxiliary request 1 (no) - auxiliary request 2 (yes)

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G 0002/98

Catchword:

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Beschwerdekammern

Boards of Appeal

Chambres de recours

Boards of Appeal of the
European Patent Office
Richard-Reitzner-Allee 8
85540 Haar
GERMANY
Tel. +49 (0)89 2399-0
Fax +49 (0)89 2399-4465

Case Number: T 1429/17 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 7 September 2021

Appellant: (Patent Proprietor)	Commonwealth Scientific and Industrial Research Organisation Black Mountain Science and Innovation Park Clunies Ross Street Acton ACT 2601 (AU)
Representative:	Ernest Gutmann - Yves Plasseraud S.A.S. c/o Plasseraud IP 66, rue de la Chaussée d'Antin 75440 Paris Cedex 09 (FR)
Appellant: (Opponent)	BASF SE 67056 Ludwigshafen (DE)
Representative:	Maiwald Patent- und Rechtsanwaltsgesellschaft mbH Elisenhof Elisenstraße 3 80335 München (DE)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 13 April 2017 concerning maintenance of the European Patent No. 1756280 in amended form.**

Composition of the Board:

Chairwoman	G. Alt
Members:	A. Chakravarty
	K. Kerber-Zubrzycka

Summary of Facts and Submissions

I. European patent No. 2 421 550, entitled "*Synthesis of long-chain polyunsaturated fatty acids by recombinant cells*", was granted on European patent application No. 1 756 280.

II. In an interlocutory decision, the opposition division decided that the patent as amended according to auxiliary request 1 and the invention to which it related met the requirements of the EPC. The opposition division also held that the main request did not meet the requirements of Article 123(2) EPC.

III. Both the patent proprietor and the opponent filed appeals against the interlocutory decision of the opposition division and will respectively be referred to as appellant I and appellant II in this decision.

IV. With their statement of grounds of appeal, appellant I submitted sets of claims of a main and four auxiliary requests which corresponded to the sets of claims of the main and auxiliary requests 1 to 4 filed on 9 January 2017 in the proceedings before the opposition division.

V. With their statement of grounds of appeal, appellant II submitted documents D39 to D52.

VI. Appellant I filed a reply to the statement of grounds of appellant II. With this reply they filed sets of claims of auxiliary requests 2 and 3. Previous auxiliary requests 2 to 4 were renumbered as auxiliary requests 4 to 6. They also filed documents D53 and D54.

VII. Oral proceedings before the board took place as requested by the parties. At these oral proceedings appellant I withdrew the main request with auxiliary request 1 becoming the *de facto* main request and auxiliary requests 2 and 3 becoming the *de facto* auxiliary requests 1 and 2. For ease of reference, the requests were not renumbered.

VIII. Appellant II made submissions on lack of inventive step in view of a combination of the disclosure in documents D21 and D8. The submissions were objected to by appellant I pursuant to Article 13(2) RPBA 2020. The board decided not to take these submissions into account.

IX. Appellant II raised an objection pursuant to Article 123(3) EPC.The board decided not to take the objection into account since the requirements of Article 13(2) RPBA 2020 were not fulfilled.

X. At the end of the oral proceedings the chair announced the decision of the board.

XI. In this decision reference to auxiliary request 1 is a reference to the *de facto* main request, while auxiliary request 2 and auxiliary request 3 are the *de facto* auxiliary requests 1 and 2, respectively.

XII. Claim 1 of auxiliary request 1 reads:

"1. A recombinant plant cell which synthesises EPA, comprising more than one heterologous polynucleotide, wherein said polynucleotides encode:
a) a Δ6 desaturase, a Δ6 elongase and a Δ5 desaturase; or

b) a Δ5/Δ6 bifunctional desaturase and a Δ5/Δ6 bifunctional elongase;
wherein the more than one polynucleotides are operably linked to one or more promoters that are capable of directing expression of said polynucleotides in the cell, wherein the enzymes encoded by said polynucleotides comprise at least one desaturase which is able to act on an acyl-CoA substrate, and wherein the synthesis of EPA requires the sequential action of said enzymes".

Claim 1 of auxiliary request 2 differs from claim 1 of auxiliary request 1 only in that in the last sentence, "comprise at least one desaturase" is replaced by "comprise a Δ6 desaturase".
Claim 1 of auxiliary request 3 reads:

"1. A recombinant plant cell which synthesises EPA, comprising more than one heterologous polynucleotide, wherein said polynucleotides encode a Δ5/Δ6 bifunctional desaturase and a Δ5/Δ6 bifunctional elongase, wherein the more than one polynucleotides are operably linked to one or more promoters that are capable of directing expression of said polynucleotides in the cell, wherein the enzymes encoded by said polynucleotides comprise at least one desaturase which is able to act on an acyl-CoA substrate, and wherein the synthesis of EPA requires the sequential action of said enzymes".

XIII. The following documents are referred to in this decision:

D1: US 60/564 627 (earlier application from which the patent in suit claims the 1st priority date).

D2: US 60/613 861 (earlier application from which the patent in suit claims the 2nd priority date)

D8: Domergue F. *et al.* (2003), "*Acyl Carriers Used as Substrates by the Desaturases and Elongases Involved in Very Long-chain Polyunsaturated Fatty Acids Biosynthesis Reconstituted in Yeast*", The Journal Of Biological Chemistry, Vol. 278, No. 37, p. 35115-3S126.

D10: Hastings N. *et al.* (2001), "*A vertebrate fatty acid desaturase with Δ5 and Δ6 activities*", PNAS, Vol. 98, No. 25, p. 14304-14309.

D21: Abbadi A. *et al.* (2004), "*Biosynthesis of Very-long-Chain Polyunsaturated Fatty Acids in Transgenic Oilseeds: Constraints on Their Accumulation*", The Plant Cell, Vol. 16, p. 2734-2748.

XIV. The arguments of appellant I relevant to the decision are summarised as follows (as regards the numbering of the requests see section XI., above):

Auxiliary request 1

Priority (Article 87(1) EPC)

Claim 1(a)

The opposition division had been incorrect to consider that sub-part (a) of claim 1 of auxiliary request 1 had no right to the first priority date. Contrary to the opposition division's finding, the feature that at least one of the desaturases was able to act on an acyl-CoA substrate was derivable from document D1.

In fact, document D1 disclosed the ability of a desaturase to act on an acyl-CoA substrate in the context of a use in yeast cells in claim 29, as well as in the discussion at page 8, line 32 to page 9, line 6.

Example 5 of document D1 provided the basis for extending this general teaching concerning desaturases acting on acyl-CoA substrates from yeast to plants. The general discussion in Example 5 made it clear that the same problems of substrate requirements of the desaturase and elongase enzymes occur in plants and in yeast. Indeed, page 53, lines 25 to 29 of document D1 formulated the advantage of the strategy devised by the inventors which was that both the desaturase and the elongase had activity on acyl-CoA substrates in the acyl-CoA pool.

Document D1 therefore disclosed, in functional terms, the activities that a desaturase and an elongase must have in order to improve the efficiency of the synthesis of long-chain polyunsaturated fatty acids (LC-PUFAs) in a plant cell. This functional definition of the desaturase and elongase enzymes would be considered as distinct from the vertebrate origin of the desaturase or the *C. elegans* origin of the elongase. Indeed, document D1 contained a general teaching, at page 9, lines 1 to 6, which defined the "*desaturases able to act on an acyl-CoA substrate*" as a group of desaturases, regardless of their organism of origin.

Inventive step (Article 56 EPC)

Claim 1(a)

Over document D21 alone

Assuming, for the sake of argument, that the subject-matter of the claim was not entitled to the first priority date, then document D21 could represent the closest prior art for the claimed subject-matter. It disclosed transgenic plants expressing different desaturases (Δ5 and Δ6) from fungi, algae, mosses and plants and a Δ6-elongase from *C. elegans*, each under the control of up to three different seed-specific promoters. Some of the transgenic plants, in particular linseed, produced eicosapentaenoic acid (EPA) at up to 1%.

The difference between the claimed subject-matter and the closest prior art was that at least one of the desaturases was able to act on an acyl-CoA substrate.

The technical effect of this difference was that the production of EPA was more efficient. In view of this, the problem to be solved by the claimed subject-matter was the provision of a plant cell with an improved efficiency of EPA production.

The claimed plant cells represented a solution to this problem that was not obvious in view of the disclosure in document D21. The conclusion reached in the decision under appeal on inventive step of the subject-matter of claim 1(a) was correct.

Contrary to the view of appellant II, document D21 taught away from the claimed subject-matter. Three

potential solutions were suggested in Figure 11 and its legend to overcome the Δ6-elongation bottleneck disclosed therein, of which the claimed subject-matter corresponded to strategy "B".

However, the skilled person reading document D21 would not have employed this strategy because it relied on the use of of animal Δ5 and Δ6 desaturases. As correctly noted by the opposition division in the decision under appeal, document D21 disclosed that these enzymes were in some instances less efficient than plant enzymes. Thus, a skilled person wishing to generate a transgenic plant producing EPA would have been cautious with regard to animal and fungal enzymes and would have favoured the use of plant enzymes over non-plant enzymes (i.e. animal and fungus enzymes).

Moreover, the skilled person would have preferred strategy "C" (employing the Δ9-elongase "alternative" pathway) to strategy "B". As correctly noted by the opposition division, strategy "C" would have been pursued by the skilled person, as it had already been successfully implemented in plants.

Another factor for the skilled person was their expectation of success when considering implementing strategy "B". As could be seen from Figure 10 in document D21 and as was confirmed on page 2735, left column, previous experiments to change fatty acid profiles of plant reserve-triacylglycerols (TAG) by seed-specific expression of various enzymes had shown that success could not be reliably predicted. The skilled person could but would not have implemented strategy "B" because the expectation of success of this strategy was too low.

Auxiliary request 2

Priority (Article 87(1) EPC) and Inventive step (Article 56 EPC)

Claim 1(a)

The arguments given for claim 1(a) of auxiliary request 1 applied equally.

Auxiliary request 3

Inventive step (Article 56 EPC)

Claim 1

Over document D21 as closest prior art

If the claimed subject-matter were held not to validly claim priority from document D1, document D21 could represent the closest prior art for the claimed invention.

The plants and plant cells claimed differed from those disclosed in document D21 in that they expressed a heterologous Δ5/Δ6 bifunctional desaturase rather than a separate monospecific Δ5 desaturase and a monospecific Δ6 desaturase. Moreover, the bifunctional desaturase in the claimed plant cells was able to act on acyl-CoA substrates, whereas the monospecific desaturases in the cells disclosed in document D21 were not able to act on acyl-CoA substrates.

There were two separate technical effects. The ability of the enzymes to act on acyl-CoA substrates resulted in an improved efficiency of EPA production in the

claimed plant cell. The use of a Δ5/Δ6 bifunctional desaturase had the effect of using fewer transgenes in the transgenic plants.

There were therefore two partial problems to be solved. The technical problem solved by the ability of the desaturase to act on acyl-CoA substrates was the provision of a plant cell with an improved efficiency of EPA production. The problem solved by the use of fewer transgenes was, at least, increased consumer acceptance.

The claimed subject-matter was not an obvious solution to either of these problems. Firstly, the use of at least one desaturase able to act on an acyl-CoA substrate to improve the production efficiency of EPA in a plant cell, was not suggested in document D21 (see the arguments presented for auxiliary request 1).

Secondly, there was no pointer in document D21 to change from using two monospecific desaturases to a single bifunctional desaturase. In fact, there was no mention of bifunctional desaturases at all in that document.

Although document D10 did disclose a bispecific desaturase from zebrafish, the skilled person would not have consulted this document, there being nothing to guide them there. Even if they had consulted document D10, they would have been unsure about whether or not a bifunctional desaturase could effectively replace the two monospecific enzymes used in document D21, especially considering that the two desaturase activities were required at different points in the biosynthesis pathway.

XV. The arguments of appellant II relevant to the decision are summarised as follows (as regards the numbering of the requests see section XI., above):

Auxiliary request 1

Priority (Article 87(1) EPC)

Claim 1

Neither one of the alternatives of claim 1 could validly claim priority from the first priority document, D1. As correctly noted in the decision under appeal, document D1 in neither the claims nor the description disclosed a plant cell comprising any desaturase able to act on an acyl-CoA substrate with respect to LC-PUFA synthesis in cells other than yeast cells.

The opposition division in the decision under appeal had also correctly held that there was no disclosure in Example 5 of document D1 of the features of claim 1(a). The disclosure in this example was limited to a particular situation, where a desaturase from zebrafish and a Δ6 PUFA elongase from *C. elegans* were expressed in a plant cell.

Inventive step (Article 56 EPC)

Claim 1(a)

Over document D21 alone

In view of a lack of a valid claim to priority from document D1, document D21 could be taken to represent the closest prior art for the claimed subject-matter.

The difference between the relevant disclosure in the closest prior art document and the claimed subject-matter was the use of at least one desaturase able to act on an acyl-CoA substrate. However, there was no evidence that this difference resulted in any improvement in eicosapentaenoic acid (EPA) production over the entire scope of the claim. The problem to be solved was therefore the provision of another plant cell synthesising EPA.

Document D21 referred to a bottleneck responsible for the low production of EPA in the transgenic plants disclosed therein (see page 2744, by column, last paragraph). The claimed solution was directly disclosed in document D21, namely the adapted Δ6 pathway disclosed in panel "B" of Figure 11 and its legend which avoided the need for shuttling between pathways by using an acyl-CoA dependent desaturase.

The opposition division was mistaken in concluding that document D21 taught away from using strategy "B" of Figure 11. The opposition division had reasoned that, from the entire disclosure of document D21, the skilled person would have been discouraged from using animal enzymes and would therefore not have pursued the adapted Δ6 prior pathway which used an desaturases "so far only known from mammals" and instead would have used the already successfully used strategy "C" of Figure 11, i.e. the alternative Δ9 pathway.

There were several flaws in this reasoning. The passage in document D21 referred to in the decision under appeal which allegedly discouraged the skilled person from adopting strategy "B" came after the description of the results achieved when expressing construct C in tobacco and linseed. This disclosure was to be seen in

the context of the experiments conducted by the authors and was not a teaching that, in general, animal enzymes were less efficient in plants and should therefore not be used.

In any case, this discussion of the results observed for the different constructs would not have discouraged the skilled person from pursuing the strategy "B". This was evident from the discussion and conclusion reached by the authors. Despite their observation that the used plant enzymes were more efficient than the animal and fungal enzymes used in the selected host, the authors explained that one strategy was the use of animal desaturases to overcome the identified bottleneck. That this strategy was considered equal to the other strategies discussed for production of EPA in plants was also evident from Figure 11.

Document D21 did not indicate a preference for Strategy "C".

A further consideration was that both Strategies "A" and "C" set out in Figure 11 of document D21 would have been less preferred by the skilled person since they required the introduction of an exogenous LPCAT (indicated by the fact that LPCAT was drawn inside the respective boxes in Figure 11). However, no such enzyme was available to the skilled person at the relevant date. In contrast, Strategy "B" utilised endogenous LPCAT (illustrated by th fact that it was drawn outside the box), leading to the skilled person having a preference for strategy "B".

Auxiliary request 2

Claim 1

The subject-matter of claim 1 encompassed the subject-matter of claim 1(a) of auxiliary request 1. The arguments given for claim 1 of auxiliary request 1 applied equally.

Auxiliary request 3

Inventive step (Article 56 EPC)

Claim 1

The difference between the disclosure in the closest prior art document D21 and the claimed subject-matter was that bifunctional enzymes were used and that at least one desaturase was able to act on an acyl-CoA substrate.

However, there was no technical effect achieved by using desaturases able to act on acyl-CoA substrates, as set out for auxiliary request 1. The effect of using a bifunctional enzyme was that fewer constructs needed to be transformed into the plants, i.e. that a less complex transgenic plant cell was provided. The problem based on this distinguishing feature was therefore the provision of a plant cell with fewer transgenes.

The skilled person knew that the use of fewer transgenes was highly desirable in production of transgenic plants. Document D10 disclosed the isolation of a Δ6/Δ5 bifunctional desaturase from zebrafish, the enzyme that was used in Example 5 of the patent. The skilled person would therefore have used the known

bifunctional enzyme to solve the problem of providing a plant cell with fewer transgenes.

XVI. Appellant I requested that

- the decision under appeal be set aside and that the patent be maintained on the basis of the set of claims of auxiliary request 1 (as the main request) or alternatively, on the basis of the set of claims of one of auxiliary requests 2 to 6.
- documents D39 to D52, filed by the opponent with their statement of grounds of appeal, not be admitted into the appeal proceedings.
- documents D53 and D54 be admitted into the appeal proceedings.

XVII. Appellant II requested that

- the decision under appeal be set aside and that the European patent No. 1 756 280 be revoked.
- documents D39 to D52, filed with the statement of grounds of appeal, be admitted into the appeal proceedings.

Reasons for the Decision

1. The appeals comply with Articles 106 to 108 and Rule 99 EPC and are admissible.

Auxiliary request 1

Priority (Article 87(1) EPC)

Claim 1(a)

2. The filing date of the patent in suit is 22 April 2005 and the filing date of the earliest document from which priority is claimed, document D1, is 22 April 2004, while the filing date of the second document from which priority is claimed, document D2, is 27 September 2004. Document D21 was published 17 September 2004. It is prior art according to Article 54(2) EPC only for subject-matter not entitled to the earliest priority date. It is therefore necessary to determine if the claimed subject-matter is entitled to claim priority from document D1.

3. According to Article 87(1) EPC, a European patent application and the resulting patent may validly claim the right of priority from a previous earlier application if both relate to "the same invention". The concept of "the same invention" expressed in Article 87 EPC has been interpreted by the Enlarged Board of Appeal in decision G 2/98 (OJ EPO 2001, 413, point 9 of the reasons) as meaning subject-matter which the person skilled in the art can derive directly and unambiguously, using common general knowledge, from the previous application as a whole.

4. In view of the submissions of the parties and of the decision under appeal, the contentious issue is whether or not a recombinant plant cell which synthesises eicosapentanoic acid (EPA), comprising heterologous polynucleotides encoding either:

a) a Δ6 desaturase, a Δ6 elongase and a Δ5 desaturase (the subject-matter of claim 1(b)); or

b) a Δ5/Δ6 bifunctional desaturase and a Δ5/Δ6 bifunctional elongase (the subject-matter of claim 1(b) wherein in both cases, the enzymes encoded by said polynucleotides comprise at least one desaturase which is able to act on an acyl-CoA substrate,

is directly and unambiguously derivable from the disclosure in document D1.

5. For a disclosure of a recombinant plant cell capable of producing EPA and comprising more than one heterologous polynucleotide as defined in claim 1(a) and in claim 1(b), appellant I relied on claims 1, 10, 36 and 43, as well as the disclosure on page 46, lines 8 and 9 and page 56 of document D1.

6. Claim 1 of the document D1 relates to a method of producing a cell, i.e. not a plant cell specifically, capable of synthesising a long-chain polyunsaturated fatty acid (LC-PUFA), i.e. not EPA in particular. It does not disclose the combination of enzymes of either (a) a Δ6 desaturase, a Δ6 elongase and a Δ5 desaturase or (b) a Δ5/Δ6 bifunctional desaturase and a Δ5/Δ6 bifunctional elongase. It does not mention acyl-CoA substrate specificity. Claim 10, of document D1 is dependent on claim 1 and specifies that "*LC-PUFA is eicosapentanoic acid (EPA)*". Claim 36 differs from

claim 1 only in that it refers to "*a cell with an enhanced capacity to synthesize a long chain polyunsaturated fatty acid (LC-PUFA)*" instead of "*a cell*". Claim 43 is for "*A transgenic plant comprising at least one cell produced by a method according to any one of claims 1 to 28, 35 or 36*".

7. From the above cited passages, it is apparent that none of the claims of document D1 alone or in combination discloses the subject-matter of either claim 1(a) or claim 1(b). Subject-matter defined by the combination of features of claim 1(a) or 1(b) is also not disclosed in the description of document D1.

8. As a basis for the combination of enzymes set out in claim 1(a) or 1(b) in combination with the feature "wherein the enzymes encoded by said polynucleotides comprise at least one desaturase which is able to act on an acyl-CoA substrate", appellant I relied in particular on page 56 of document D1.

9. The first passage relied on was lines 14 to 21 of document D1 which reads:

"*Synthesis of LC-PUFAs such as EPA and DHA in cells such as plant cells by the Δ6 desaturation pathway requires the sequential action of PUFA desaturases and elongases. The required desaturases have Δ6, Δ5 and Δ4 desaturating activity, in that order, and the required PUFA elongases have elongating activity on Δ6 and Δ5 substrates. This conventional pathway operates in algae, mosses, fungi, diatoms, nematodes and some freshwater fish (Sayanova and Napier, 2004). The PUFA desaturases from algae, fungi, mosses and worms are selective for desaturation of fatty acids esterified to the sn-2 position of phosphatidylcholine (PC) while the*

PUFA elongases act on fatty acids in the form of acyl-CoA substrates represented in the acyl-CoA pool of tissues. In contrast, vertebrate Δ6 desaturases have been shown to be able to desaturate acyl-CoA substrates (Domergue et al 2003)".

10. However, this passage merely references the known different specificities of PUFA desaturases from algae, fungi, mosses and worms in contrast to the vertebrate Δ6 desaturases and is not a disclosure of the combination of enzymes set out in claim 1(a) or 1(b) with the feature "wherein the enzymes encoded by said polynucleotides comprise at least one desaturase which is able to act on an acyl-CoA substrate".

11. The second passage relied on is that on page 56 from line 25 to line 35 which reads:

"*The strategy described above of using a vertebrate desaturase, in this example a Δ5/Δ6 desaturase from zebra fish, with a Δ6 PUFA elongase from C.elegans had the advantage that both the desaturase and the elongase have activity on acyl-CoA substrates in the acyl-CoA pool. This may explain why this strategy was more efficient in the synthesis of LC-PUFA. Furthermore, using a bifunctional desaturase displaying dual Δ5/Δ6 desaturase activities allowed the synthesis of EPA the the action of only 2 genes instead of the 3 genes used by other researchers .(Beaudoin et 2000, Domergue et al, 2003)*".

12. This passage, which forms part of Example 5, does not disclose the combination of enzymes set out in claim 1(a) or 1(b) in combination with the feature "wherein the enzymes encoded by said polynucleotides comprise at least one desaturase which is able to act on an acyl-

CoA substrate" either. Instead, it discloses a particular embodiment in which a Δ5/Δ6 desaturase from zebra fish and a Δ6 PUFA elongase from *C. elegans* are co-expressed in plant cells. This limitation to a particular embodiment is also reflected in the heading of the example which reads "*Genetic construct for co-expression of the zebrafish Δ6/ Δ5 desaturase and C .elegans elongase in plant cells".* In view of this, the board is not persuaded by the appellant's argument that the reference to a "*strategy [...] of using a vertebrate desaturase*" imparts generality on the use of vertebrate desaturases outside of the specific example. Instead, it is apparent for the overall context that the "*strategy*" referred to was the specific strategy employed in the example. Moreover, this specific example cannot provide a disclosure of the combination of three monofunctional enzymes as set out in claim 1(a), since it relates to the use of a single, bifunctional Δ5/Δ6 desaturase from zebrafish and a Δ6 PUFA elongase from *C. elegans*.

13. There is no disclosure of the subject-matter of claim 1(b) in this passage either, since the passage relates to a combination of enzymes from specific source organisms, while the claim is not so limited.

14. In view of the above considerations, the board concludes that the subject-matter of claim 1 is not directly and unambiguously disclosed in document D1 and is therefore not entitled to claim priority from this document.

15. Thus, document D21 is prior art for the subject-matter of claim 1 pursuant to Article 54(2) EPC.

Inventive step (Article 56 EPC)

Claim 1(a)

Closest prior art

16. Document D21 is an article entitled "*Biosynthesis of Very-Long-Chain Polyunsaturated Fatty Acids in Transgenic Oilseeds: Constraints on Their Accumulation*".

It discloses the "*Seed-specific expression in transgenic tobacco (Nicotiana tabacum) and linseed (Linum usitatissimum) of cDNAs encoding fatty acyl-desaturases and elongases, absent from all agronomically important plants, resulted in the very high accumulation of Δ6-desaturated C18 fatty acids and up to 5% of C20 polyunsaturated fatty acids, including arachidonic and eicosapentaenoic acid*" (see abstract).

17. The document reports experiments on the production of arachidonic acid (ARA) and EPA in seeds of transgenic higher plants using cDNAs encoding two regio-specifically different desaturases (Δ6 and Δ5) and a Δ6-elongase, each under the control of up to three different seed-specific promoters as heterologous coding sequences.

18. The best combination tested included the Δ6 desaturase from the diatom *P. tricornutum*, the Δ6 elongase from the moss *P. patens* and the Δ5 desaturase from *P. tricornutum* and resulted in tobacco seeds with up to 2% ARA and linseed seeds with up to 1.5% ARA and 1 % EPA (see page 2736, left column). The fatty acid composition of a seed mixture of five independent

plants in Table 1 shows that an average of 0.8% EPA was produced in linseed.

19. The board, in agreement with the opposition division in the decision under appeal, considers that document D21, and in particular the transgenic plants producing EPA disclosed therein, can represent the closest prior art for the claimed subject-matter. These plants (and their cells) differ from those claimed in the specificity of at least one of the desaturases, which in contrast to those in the plants disclosed in document D21, is able to act on an acyl-CoA substrate.

The technical problem

20. There was disagreement between the parties about whether or not the above defined difference was associated with a technical effect. Appellant I was of the view that the technical effect of the difference was improved EPA production efficiency in the claimed plant cell. Appellant II was of the view that the difference was not associated with a technical effect, at least over the entire scope of the claim.

21. On the basis of the evidence before the board, it is not possible to conclude that the effect of improved efficiency of EPA production is valid over the entire scope claimed. This is because no data directly comparing the efficiency of EPA production in the plants/plant cells of the closest prior art with those claimed has been provided. Indeed, the patent itself has no example of a recombinant plant cell corresponding to claim 1(a), where the heterologous polynucleotides encode three separate monofunctional enzymes. The board therefore considers that no effect beyond the ability to synthesise EPA can be ascribed to

the technical difference between the plants/plant cells disclosed in document D21 and those claimed.

22. In view of the above, the technical problem to be solved by the plant cells of claim 1 is the provision of alternative recombinant plant cells which synthesise EPA.

Obviousness

Claim 1(a)

23. In deciding on obviousness, the question to be answered is whether or not the skilled person, faced with the above formulated technical problem and starting from the EPA producing plants (and their cells) disclosed in document D21, would have provided the claimed plant cells comprising heterologous polynucleotides encoding a Δ6 desaturase, a Δ6 elongase and a Δ5 desaturase wherein the enzymes encoded by said polynucleotides comprise at least one desaturase which is able to act on an acyl-CoA substrate.

24. As set out above, document D21 discloses transgenic tobacco and linseed plants, engineered to express different heterologous desaturases (Δ6 and Δ5) and a Δ6-elongase. These plant cells produced up to 5% C20 PUFA (see abstract). ARA accumulated up to 2% in tobacco, whereas in linseed, both ARA (up to 1.5%) and EPA (up to 1 %) were found (see Figure 4, Table 1).

Under the heading "*Why Not More ARA and EPA in the Transgenic Seeds: Is the Elongase Step Limiting?*" (see page 2726) it is disclosed that "*Our data with linseed indicate that after desaturation, Δ6-C18-PUFA are likely to be channeled from PC directly to other*

lipids, preventing their exchange with the acyl-CoA pool and efficient subsequent elongation. Accordingly, the introduction of lipid linked Δ6-desaturation produc ts into the acyl-CoA pool represents the most severe bottleneck for the production of VLCPUFA in transgenic linseed" (see page 2744, left column).

25. Furthermore, under the heading "*Strategies to Improve VLCPUFA Synthesis in Transgenic Plants*" on page 2745, it is stated that *"The identification of the bottleneck in our approach raises additional interest in alternative strategies for VLCPUFA biosynthesis, as briefly outlined here (Figure 11). For example, the alternative shown in Figure 11 B makes use of animal Δ6- and Δ5- front end desaturases that have been shown to use acyl-CoA as substrates (Okayasu et al., 1981; lrazu et al., 1993). With such enzymes, the conversion of linoleic or α-linolenic acid to ARA or EPA would be accomplished exclusively on the acyl-CoA track and thus avoid the switching between lipids and acyl-CoA (Figure 11 B)*".

26. Document D21 (see Figure 11) therefore suggests three different strategies (A, B and C) that can be used to generate VLC-PUFA. These are

"(A) The lipid-linked desaturation pathway was followed by our approach and requires both forward and reverse reactions of a Δ6-specific LPCAT, which is the limiting step in linseed."

"(B) The acyl-CoA pathway, where both the elongation and desaturation occur in the acyl-CoA pool, requires acyl-CoA front-end desaturases so far only known from mammals" and

"*(C) An alternative pathway relying on the initiating Δ9-elongation step in the acyl-CoA pool followed by lipid-linked desaturations has been verified in leaves*" (see legend to Figure 11).

Strategy "B" corresponds to subject-matter of claim 1(a).

27. In the board's view, the skilled person could and would have adopted any of strategies "A", "B" and "C" as a solution to the above formulated technical problem. The skilled person seeking to produce an alternative recombinant plant cell which synthesises EPA and adopting strategy "B", would have produced a plant cell in which the heterologous polynucleotides encode a Δ6-desaturase, a Δ6-elongase and a Δ5-desaturases that act in the acyl-CoA pool, where the heterologous polynucleotides encoding the Δ6- and Δ5-desaturases encode animal Δ6- and Δ5-front end desaturases.

28. Appellant I argued that document D21 taught away from the use of strategy "B" for a number of reasons:

1) Figure 10 and the corresponding part of page 2735, (see left column) showed that the acyl-CoA pool was at the centre of a complex pathway. The skilled person would have hesitated to interfere with this pathway.

2) The skilled person would have implemented strategy "C" in preference to strategy "B" because it used a Δ9 elongase approach that had already been successfully implemented in plants, whereas expression of animal desaturases was untried in plants.

3) The skilled person would have been hesitant to use animal enzymes because they were identified as less efficient in document D21.

29. The board is not persuaded by these arguments, for several reasons:

Firstly, strategy "B" is explicitly suggested in document D21. This suggestion is not accompanied with any provisos and therefore carries persuasive weight.

Secondly, appellant I's arguments which aim to demonstrate that document D21 teaches away from the claimed subject-matter are based on a reading of document D21 which is, in the board's view, not one which the skilled person would derive from that document. The passage in document D21 on page 2735, reading "*Previous experiments to change the fatty acid profiles of plant reserve triacylglycerols (TAG) by seed-specific expression of various enzymes have shown that the success of these manipulations cannot be reliably predicted*" was cited, in conjunction with Figure 10, as evidence that the skilled person would have been discouraged from adopting strategy "B" due to unpredictability. However, the passage in question is part of a summary of the work done before the experiments reported in document D21. Indeed, document D21 itself discloses "*Seed-specific expression in transgenic tobacco (Nicotiana tabacum) and linseed (Linum usitatissimum) of cDNAs encoding fatty acyl-desaturases and elongases, absent from all agronomically important plants, resulted in the very high accumulation of Δ6-desaturated C18 fatty acids and up to 5% of C20 polyunsaturated fatty acids, including arachidonic and eicosapentaenoic acid*" (see abstract).

Appellant II, at oral proceedings before the board, argued that both Strategies "A" and "C" set out in Figure 11 of document D21 would have been less preferred by the skilled person since they required the introduction of exogenous LPCAT (indicated by the fact that LPCAT was drawn inside the respective boxes in Figure 11). However, no such enzyme was known to the skilled person. In contrast, Strategy "B" utilised endogenous LPCAT (illustrated by th fact that it was drawn outside the box). In the board's view this argument has some merit, since not needing to identify a suitable LPCAT makes strategy "B" easier to implement and therefore more attractive.

30. In any case, even if the board had been persuaded that the skilled person would have preferred strategy "A" or "C" over strategy "B", the skilled person would not have dismissed this strategy entirely, leaving strategy "B" as one of three suitable and thus obvious alternatives. A selection from several obvious alternatives does not impart an inventive step (cf. Case Law of the Boards of Appeal of the European Patent Office, 9th edition 2019, I.D.9.19.10).

31. In summary, the skilled person starting from document D21, which identified "*the introduction of lipid linked Δ6-desaturation products into the acyl-CoA pool*" as "*the most severe bottleneck for the production of VLCPUFA*" (see page 2744, right column) and seeking a solution to the technical problem, would have adopted one of the "*alternative strategies for VLCPUFA biosynthesis*", including strategy "B", outlined therein. This would have led them to the subject-matter of claim 1(a), which therefore lacks an inventive step.

32. Thus, auxiliary request 1 is not allowable.

Auxiliary request 2

Inventive step (Article 56 EPC) - claim 1

33. As noted in Section XII., claim 1 of auxiliary request 2 differs from claim 1 of auxiliary request 1 only in that, in the last sentence of the latter "comprise at least one desaturase", is replaced by "comprise a Δ6 desaturase". The claim nevertheless encompasses the subject-matter of claim 1(a) of auxiliary request 1 which was found to be obvious above. Thus, the conclusions reached for claim 1(a) of auxiliary request 1 apply equally.

Auxiliary request 3

Novelty (Article 54 EPC)/Clarity and support in the description (Article 84 EPC)

34. The only objection to auxiliary request 3 raised by appellant II was under Article 56 EPC. The board has no reason not to hold that the subject-matter of auxiliary request 3 meets the requirements of Article 54 EPC and of Article 84 EPC.

Inventive step (Article 56 EPC) - claim 1

35. Claim 1 of auxiliary request 3 corresponds to claim 1(b) of auxiliary request 1 and specifies that the two desaturase activities are provided by a Δ6/Δ5 bifunctional desaturase.

36. The board is not persuaded that the conclusion on inventive step reached for the subject-matter of claim 1(a) of auxiliary request 1 applies to the subject-matter of claim 1 of the present claim request.

While the technical problem remains the same as formulated for claim 1 of auxiliary request 1 (see point 22. above), the considerations on obviousness differ.

37. The skilled person starting from transgenic plants/ plant cells capable of synthesising EPA disclosed in document D21 representing the closest prior art, would have in document D21 found no suggestion to employ bifunctional desaturases.

38. The transgenic plants/plant cells disclosed in document D21 contain monospecific desaturases (see Figure 2) and the skilled person seeking an alternative plant/plant cell would, as set out in points 16. to 32., have turned to one of the strategies illustrated in Figure 11 and discussed on page 2745 (left column).

39. Although a bifunctional Δ6/Δ5 bifunctional desaturase from zebra fish is disclosed in document D10, there is nothing in document D21 that would have given the skilled person any suggestion to use it. The reverse is also true: Document D10 makes no mention of the potential utility of the zebrafish desaturase disclosed therein in the synthesis of PUFAs in plants.

40. In view of this, the board considers that the skilled person could, but would not, have used the bifunctional Δ6/Δ5 bifunctional desaturase from zebrafish disclosed in document D10 when seeking to implement strategy "B" suggested in document D21.

41. Appellant II suggested that the skilled person knew that the use of fewer transgenes was highly desirable in production of transgenic plants. This knowledge would have led the skilled person to use this known

bifunctional enzyme to solve the problem of providing a plant cell with fewer transgenes.

42. The board is not persuaded by this argument. Although it accepts that at the relevant date of the patent it was generally desirable to use as few transgenes as possible, this general consideration would not have outweighed the specific teaching in document D21 which suggested the use of separate enzymes for each desaturase function.

43. In view of the above considerations, the subject-matter of claim 1 is considered to meet the requirements of Article 56 EPC.

Admission of a line of argument of lack of inventive step based on a combination of document D21 with document D8 (Article 13(2) RPBA)

44. At the oral proceedings before the board, appellant II requested to present a line of argument to the effect that the subject-matter of claim 1 of auxiliary request 3 lacked an inventive step in view of a combination of the disclosure in document D21, representing the closest prior art, with the disclosure in document D8.

45. The board decided not to take this line of argument into account because it had not been made in the preceding written proceedings. It therefore represented an amendment to the appeal case of appellant II, made after notification of a summons to oral proceedings. Pursuant to Article 13(2) RPBA 2020 such an amendment is, in principle, not to be taken into account unless there are exceptional circumstances, which have been

justified with cogent reasons. No such reasons were supplied.

Admission of an objection of unallowable extension of scope pursuant to Article 123(3) EPC (Article 13(2) RPBA)

46. At the oral proceedings before the board, appellant II also requested to present a line of argument to the effect that the amendments in claim 1 of auxiliary request 3 resulted in an extension of scope vis-à-vis the patent as granted and were therefore not allowable. They stated that they had not made this argument before during the appeal proceedings and did not have cogent reasons for doing so only at this late stage.

47. The board decided not to take this line of argument into account pursuant to Article 13(2) RPBA (see point 44. above).

48. In view of the above considerations, the appeal of appellant II is not allowable and must be dismissed. The second auxiliary claim request of appellant I, i.e. that the patent be maintained on the basis of the set of claims of auxiliary request 3, as filed with the letter dated 4 January 2018 is allowable.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the opposition division with the order to maintain the patent on the basis of the set of claims of auxiliary request 3 as filed with the reply dated 4 January 2018 to appellant II's statement of grounds appeal, with a description to be adapted thereto.

The Registrar: The Chair:

I. Aperribay G. Alt

Decision electronically authenticated